FITC PFPE amide (FBPA composed of PFPE amide 1, 40 and 41). A flame dried round bottom flask (50 mL) was charged with perfluoropolyether methyl ester 39 (1.62 mL, 1.5 mmol) under an argon atmosphere. FITC-TR cadaverine (0.01 g, 0.015 mmol, 1 mol%) was dissolved in trifluoroethanol (2.0 mL) and added to PFPE oil, followed by triethylamine (0.84 mL, 6.0 mmol). The reaction mixture was allowed to stir at r.t. for 48 h protected from light. Diethylamine (1.24 mL, 12.0 mmol) was added to convert all unreacted ester end groups of PFPE into tertiary amide, and the reaction continued for 72 h at r.t. The excess diethyl amine and methanol were removed by vacuum, and the final dark yellow oil was washed once with ethanol, concentrated in vacuo and used for fluorescent nanoemulsion preparations without further purifications.

Alexa647 PFPE amide (FBPA composed of PFPE amide 1, 18 and 19). A flame dried glass vial (2.5 mL) flushed with argon, was charged with perfluoropolyether methyl ester 39 (0.086 mL, 0.08 mmol). Alexa647-TR cadaverine (0.002 g, 0.002 mmol, 2.5 mol%) was dissolved in trifluoroethanol (2.0 mL) and added to PFPE oil, followed by triethylamine (0.04 mL, 0.29 mmol). The reaction mixture was allowed to shake on a mechanical shaker at 80 rpm at r.t. for 48 h protected from light. Diethylamine (0.08, 0.77 mmol) was added to convert all unreacted ester end groups of PFPE into tertiary amide, and the reaction was allowed to proceed for 72 h at r.t. The excess diethyl amine and methanol were removed by vacuum, and the final dark yellow oil was washed with ethanol, concentrated in vacuo, and used for fluorescent nanoemulsion preparations without further purifications.

The FBPA was used for fluorescent PFPE nanoemulsion preparations by microfluidization on large scale (25mL). PFPE amides demonstrated stability and held the fluorescent dye in the PFPE oil phase during microfluidization. Fluorescence measurements showed the BODIPy-TR dye spectrum did not change after coupling to PFPE. Using the same approach, PFPE methyl ester 39 was labeled with highly fluorescent AlexaFluor 647 dye, to allow prolonged fluorescent imaging of labeled cells *in vivo*. AlexaFluor labeled cadaverine was directly coupled to PFPE methyl ester 39 at 2.5 mol% and the rest of ester end groups were converted to diethyl amide by treatment with excess diethylamine. Labeling was performed at room temperature in trifluoroethanol After the

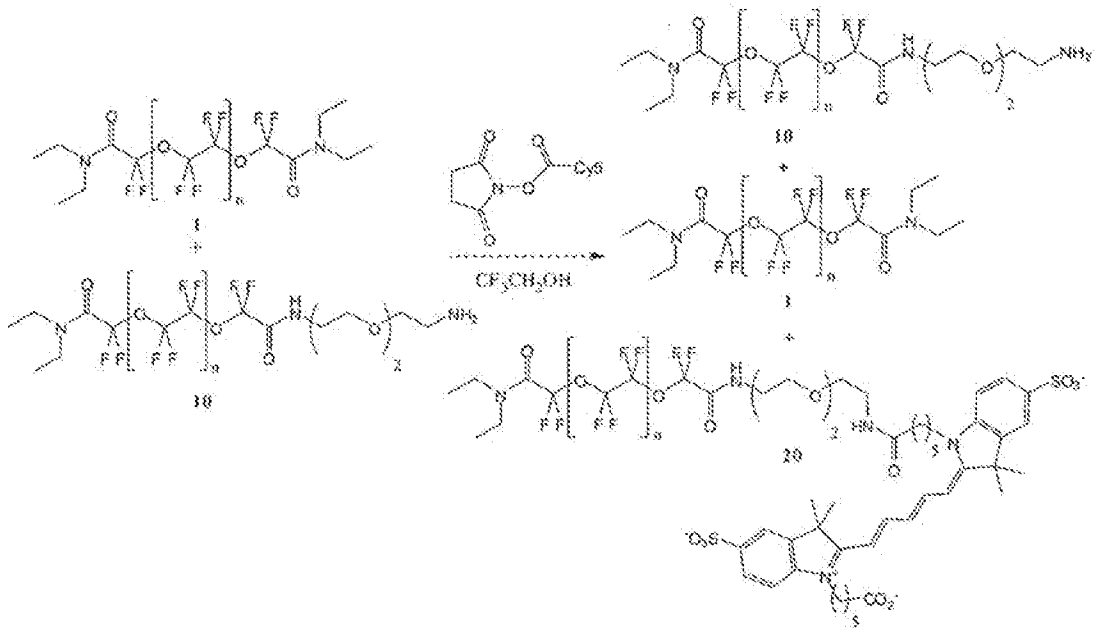
fluorescent dye was coupled, fluorescence and UV/VIS absorbance measurements of labeled PFPE were used for coupling efficiency estimates, (Figure 43A-B). . Following the same approach, the PFPE ester 39 was coupled to FITC dye via cadaverine linker at 2 mol%, while the rest of ester end groups were converted to tertiary amides with excess diethylamine (Scheme 4). In all cases the resulting product is fluorescent "blended" PFPE amide (FBPA). These oils were named "blended" because of the specific nature of fluorinated oils to stay together as its own phase. These fluorescent oils behaved as unique oil phase in all emulsion preparation experiments.

We have also explored how well PFPE oxide 1a and PFPE amide 1 blend together. We have found that PFPE oils blend at any given ratio and we used the blended PFPE oils (PFPE amide 1 and PFPE oxide 1a) for nanoemulsion preparations by microfluidization. We also successfully blended FBPA oils with PFPE oxide 1a in order to prepare highly stable fluorescent nanoemulsion on large scale. The "blended" PFPE oil approach can be and was applied to all PFPE derivatives and it was successful in most emulsion preparations. The PFPE derivatives tended to form a unique oil phase that was not mixing readily with either water or oil and rather stayed as a separate phase. This was mostly true for those "blended" oils that contained fluorescent dyes, or were made by mixing PFPE amide 1 and PFPE oxide 1a at different ratios.

By definition "blended" PFPE oil contains PFPE derivative 1 mixed with PFPE oxide 1a at volume ratios from 1/100 – 1/1. In most emulsions we used 1/9 ratio v/v. If the PFPE amide is actually FBPA, it was also blended with PFPE oxide 1a at volume ratio 1/10. These blends were used for fluorescent nanoemulsion preparations described in detail below.

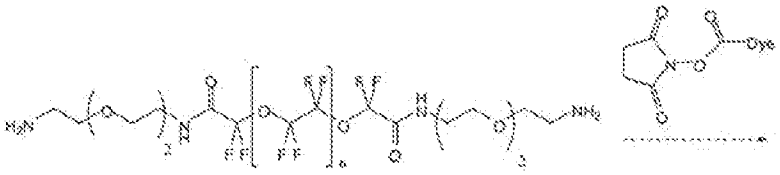
The amino-reactive dye Cy5 may be used to couple to an imaging reagent. In such instances the composition comprising a compound of formula 10 and a compound of formula 1 may be used, as shown in Scheme 5, wherein n, independently for each occurrence, represents an integer from 4 to 16.

Scheme 5:

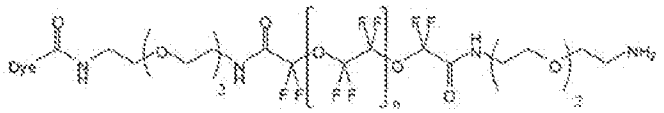


In general, any amino-reactive dye can be coupled onto an imaging reagent set forth above that contains an amine functionality. For example, compounds 27, 30, 33, or 36 may be prepared according to the proposed reactions in Scheme 6, wherein n, independently for each occurrence, represents an integer from 4 to 16. Furthermore, any of these compounds can be further blended with PFPE oxide 1a and/or PFPE amide 1 to form new FBPA blends, as described above. This takes advantage of the fact that PFPE derivatives tend to mix better with PFPE derivatives than with water or organic oils. Any of these fluorescent and non-fluorescent PFPE derivatives can be blended with each other in any combination, providing diverse set of PFPE oils available for nanoemulsion preparation and further fine tuning of final nanoemulsion MRI reagent properties.

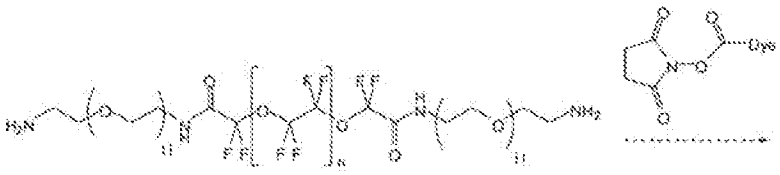
Scheme 6:



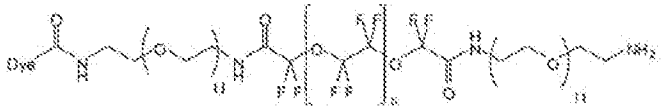
11



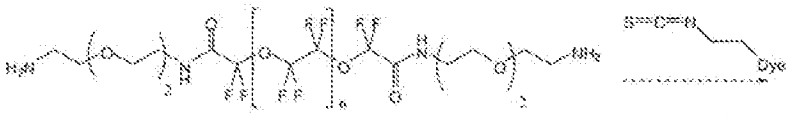
27



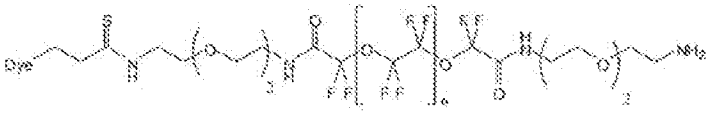
13



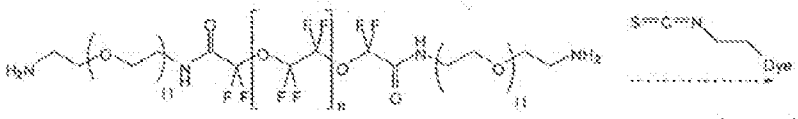
30



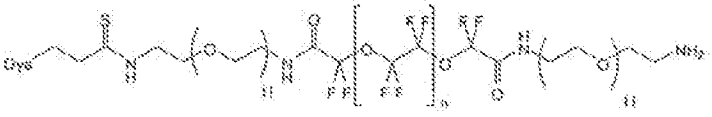
11



33



13



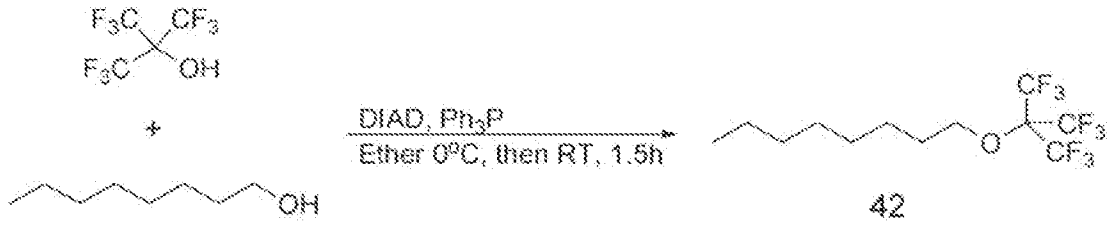
36

Due to the low concentration of dyes used for labeling PFPE modified oils, 19F NMR and MS data were not sufficient indicators for the coupling yield. We could not observe presence of the dye that was usually 1 mol% or less by NMR or MS analysis. Alternative analytical approaches were used. A combination of spectrophotometric analysis and fluorescence was used to determine the dye coupling to the PFPE. In the case of the highly

fluorescent Alexa647 dye, extremely low concentrations (0.05 mol%) were sufficient to give adequate fluorescent tagging of target cells labeled with PFPE emulsions. In the case of BODIPy-TR label, 0.5mol% was sufficient. For each dye, standard curves were constructed in trifluoroethanol to avoid solubility differences between PFPE, PFPE conjugates, and the free dye. Fluorescently labeled PFPEs were purified by solvent extraction or FluoroFlash filtration to remove unreacted dye. Then, fluorescent PFPE oil was dissolved in trifluoroethanol and both UV/VIS absorbance and fluorescence were measured. Concentration of the dye was estimated from the constructed standard curves by two independent methods, including fluorescence and UV/VIS absorbance (Figure 43A-B). It is known in the art that fluorescent dyes can change their fluorescent properties upon conjugation to macromolecules. Therefore, the absorbance and emission spectra were obtained for fluorescently labeled PFPE, compounds of formula 10 and formula 1, and compounds of formula 16 and formula 1; Fluorescence emission spectra of Alexa 647 was unaffected by the conjugation to PFPE (Figure 43C-D). Examples of analytical data, including HPLC, ^{1}H, ^{13}C, ^{19}F NMR and MALDI-TOF of selected PFPE derivatives, are presented in Figures 41-52.

Alternative Channel MRI Reagent (compound 42):

Compound **42** was prepared according to Scheme 7 and the following procedure:



42

The following procedure was modified from Jiang *et al* (36). To a solution of 1-octanol in anhydrous ether (5.25 g, 40.35 mmol) was added triphenylphosphine (11.11 g, 42.37 mmol) and the mixture stirred at room temperature for 15 minutes until the powder completely dissolved. The reaction mixture was then placed on an ice bath (0 °C) and diisopropylazodicarboxylate (8.71 mL, 44.38 mmol) was added dropwise. The addition was performed under argon atmosphere. During the addition the solution changed color to pale yellow and yellow precipitate formed. After the addition was complete, the reaction mixture was stirred for an additional 30 minutes on the ice batch and then perfluoro-*tert*-butanol (10.00 g, 42.37 mmol) was added in one portion and the resulting mixture was

stirred for 1.5 h at room temperature. The crude reaction mixture was filtered over short SiO_2 column to remove triphenylphosphine oxide precipitate. The filtrate was concentrated, redissolved in a small amount of ether and loaded on a SiO_2 column. The product was eluted with perfluorohexanes/ether (1:1 v/v) mixture and concentrated in vacuo. Removal of the unreacted perfluoro-*tert*-butanol under vacuum gave the product **42** as a clear colorless oil (8.36 g, 59.5%). ^{1}H NMR (300 MHz, d_6-acetone) δ 4.12 (t, 2H, J=6.2 Hz), 1.79 (dt, 2H, J=6.6, 6.3 Hz), 1.46-1.30 (m, 10H); ^{19}F NMR (477MHz, neat) δ −71.39 (s, 9F); ^{13}C NMR (75.6 MHz, neat) δ 120.5 (q, J=289.9 Hz), 70.3, 31.5, 25.1, 22.3; MS (ESI) *m/z* 388.5 (M^++K, 40).

2. Emulsion preparation

PFPE is a lipophobic and hydrophobic polymer, which presents challenges for emulsification. On the other hand, there is a clear advantage to these properties. PFPE oils readily mix with each other at any given ratio providing a unique fluorinated phase for nanoemulsion preparation. Blended PFPE oils are readily accessible through simple vortexing of diverse PFPE derivatives (e.g., the FBPAs described above). The blended oils may then be used for variety of emulsion preparations and in general behave as a single PFPE oil phase.

A variety of emulsification methods were extensively explored. Sonication and microfluidization processing techniques were tested, as well as low energy methods, such as thin film and vortexing. In all emulsion preparations, PFPE derivatives remained chemically unchanged. Lipids, block copolymers (e.g., Pluronics™) and polyamines were used as excipients for PFPE emulsification.

In all of the experiments using sonication, a Sonifier Cell Disruptor (Misonix Inc., Farmingdale, New York) was used at 20 watts output power and at 20 kHz. The tip of the probe is immersed into a small volume of liquid, usually 0.1-0.5 mL in an Eppendorf tube. Unless noted differently, all emulsifications were performed at room temperature.

In all of the described experiments using microfluidization, a M-110S Laboratory Microfluidizer Processor (Microfluidics, Inc., Newton, MA) was used at 40-60 psi working pressure with sample volumes in the range 10-40 mL, not excluding higher

volumes (up to 100mL). Microfluidization, homogenization, and the like can also be used for production of large batches of emulsion product exceeding 1 L.

Specific Examples of Emulsion Formulations:

PFPE amide/F68 emulsion:

Pluronic™ F68 was dissolved in aqueous buffer (HBSS, Hyclone) at 150 mg/mL. PFPE amide 1 was added at 152 mg/mL, and the mixture (250 µL) was placed in an 1.5mL Eppendorf vial and sonicated (at 20 W and 20 kHz) at room temperature for 30 seconds. The emulsion was then diluted 1:3 in 1X HBSS and the particle size was measured by dynamic light scattering in Malvern Zetasizer Nano within 1 h. The molar ratio of PFPE amide 1 to F68 was 4.6:1. The polydispersity (PDI) was 0.12 to 0.19 and the average particle size 200 nm. The critical micellar concentrations (CMC) for the PFPE amide 1 and F68 was estimated by plotting the light scattering intensity and average diameter and looking for the curve intersection, as shown in Figure 1.

PFPE/L35 emulsion:

PFPE amide 1 (24 µL, 36.5 mg, 0.02 mmol) and Pluronic™ L35 (54 µL, 57.4 mg, 0.03 mmol) were mixed in an 1.5 mL Eppendorf tube and sonicated neat for 10 s. Deionized water (122 µL) was then added and the mixture was sonicated until it turned clear (10-15 s). Emulsions prepared as above were stable at high concentrations for about 2-3 hours. If the emulsion was diluted 10-fold in water, the particle size and PDI remained constant for at least 3-5 days.

Dual Fluorescent/MRI Nanoemulsion Label – Pilot version prepared by sonication:

The first fluorescent nanoemulsion was prepared with BODIPy-TR PFPE amide by sonication. The composition containing PFPE-BODIPy amide 16 and PFPE amide 1 (1 mol% dye) was diluted in pure PFPE amide 1 at various concentrations. For a given mixture dye concentration, the PFPE/BODIPy (24 µL) was sonicated with L35 (54 µL) and water (122 µL). The presence of BODIPy-TR, a lipophylic neutral dye, in PFPE-amide oil 1 had little effect on particle size and emulsification of PFPE amide 1, as shown in Figure 2. The particle diameter was 114.1±31.4 nm and the PDI varied from 0.1 to 0.4.

BODIPy-PFPE amide 16/L35 emulsions with increasing BODIPy-TR dye concentrations (0-1 mol%) were diluted in water 10 times. The fluorescence intensity was measured in a black, flat bottom 96-well plate on TECAN plate reader using 580 nm excitation and 630 nm emission, as shown in Figure 3.

The presence of lipophilic fluorescent dye had virtually no effect on emulsification of PFPE amide 1 with L35 by sonication. The particle size and PDI were comparable, and this result indicated that uptake may not change. This is important because tissue culture protocols that are developed for labeling cells can be used for both fluorescent and non-fluorescent versions of the PFPE nanoparticles. Furthermore it is anticipated that the fluorescent analogs could be used for in vitro measurements of the key parameter Fc as part of the cell labeling protocol development or validation. Thus, the mean cell loading, measured by Fc, could be evaluated using low-cost fluorimeters, rather than expensive 19F NMR instrumentation , and the Fc result could be used for subsequent in vivo experiments using the non-fluorescent versions of the PFPE.

A nanoemulsion with Alexa647-PFPE amide was prepared following the same procedure as above. Alexa647 PFPE amide was diluted in PFPE amide to obtain final dye concentration at 0.05 mol% to PFPE, which was then emulsified with L35. The average emulsion particle diameter was determined to be 122 ± 17 nm.

Composition of PEG-PFPE-amide 14 and PFPE amide 1:

The composition comprising PEG-PFPE amide 14 and PFPE amide 1 (25μL) was sonicated in 1 mL of water (as shown in Figure 4) or 1X HBSS (as shown in Figure 5) for 30 seconds. The emulsion dilutions were then tested using DLS. Particle size of PEG-PFPE amide 14 in water was 144±17.8 nm with PDI ranging from 0.2 to 0.3. The critical micellar concentration in water 33.5mg/mL was estimated by plotting scattering light intensity and particle size (Figure 4). Particle size increased to 211±36.4 nm, with PDI ranging form 0.15 to 0.40, when PEG-PFPE was emulsified in 1X HBSS buffer. The CMC in 1X HBSS was estimated to be 30.1mg/mL, as shown in Figure 5.

Composition of PEG-PFPE-amide 10 and PFPE amide 1:

Different concentrations of the composition of PEG-PFPE-amide 10 and PFPE amide 1 were prepared by carefully diluting a composition of 33 mol % PEG-PFPE-amide 10 in PFPE amide 1. A total of 9 oil samples were prepared with decreasing concentrations of PEG-PFPE-amide 10, from 33 to 0 mol%. Each oil (25 µL) was sonicated in 125 µL of water or 1X HBSS for 10 sec, then diluted to 750 µL with external phase and sonicated for 30 sec, at the same power setting. The emulsions were then tested for particle size and zeta potential and stored at room temperature. Figures 6 and 7 shows the particle size and scattering intensity for all samples. When water was used as medium, the average particle size was 195.4±4.5 nm with PDI = 0.1-0.3, as shown in Figure 6. When 1X HBSS was used, particle size was unchanged, 193±14.5nm with PDI=0.1-0.3, as shown in Figure 7.

In both media, the amount of PEG-PFPE amide 10 in PFPE amide 1 had no effect on the particle size or polydispersity. Emulsions with less then 5 mol% PEG-PFPE amide 10 remained stable for at least four days at room temperature. These stable emulsions kept the same particle size, scattering intensity and polydispersity, as measured by DLS measurements. The same stability was observed both in water and HBSS buffer. Covalent conjugation of a very short PEG of only two ethylene oxide units can serve two purposes. First, it can serve as a linker for attachment of targeting agents and dyes. Second, it aids in emulsification by acting as both an emulsifier and a steric stabilizer of formed droplets.

Composition of PEG-PFPE-amide 10 and PFPE amide 1/L35:

The composition of PEG-PFPE-amide 10 and PFPE amide 1, with 4 mol% of 10, was compared to PFPE amide 1 alone when emulsified by sonication with L35 in a mol ratio 1:1, in 1X HBSS, following the same procedure as described above for the same composition. Data is presented in Figure 8.

When L35 was used as emulsifier, the presence of PEG-PFPE-amide 10 on PFPE amide 1 did not have significant effect on particle size. However, emulsification of PFPE amide 1 with L35 gave significantly lower particle size then PFPE amide 1 sonicated in 1X HBSS alone. These findings indicate that the presence of hydrophilic groups surrounding the PFPE amide emulsion droplet promotes the emulsion stability.

Emulsification with lipids:

Cationic lipids CTAB, DDAB, and a neutral lipid DMPC were used alone or with Pluronic™ L35 to emulsify PFPE amide 1. These lipids are expected to improve particle uptake by promoting cell membrane adherence. Lipids with positive charge are known to promote cell-particle interactions due to the negative charge of the cell membrane.

The addition of lipids required modification to the sonication methods. Lipids as solids were first dissolved in an appropriate organic solvent (e.g., chloroform or acetone), mixed with PFPE amide 1, and dried into a thin film using a stream of argon gas. The film was then vortexed with aqueous media (water or 1X HBSS buffer), heated to 50-70 °C for 15 minutes, and vortexed again while cooling to room temperature. Emulsions were then sonicated to decrease the particle size and PDI.

DMPC/PFPE amide 1 emulsion:

PFPE amide 1 solution (152 mg/mL) in trifluoroethanol (200 μL) and DMPC solution in chloroform (200 μL), containing decreasing concentrations from 112 to 0 mg/mL, were mixed by vortexing and dried into a thin film using argon stream. The film was dried under vacuum for 30 minutes at room temperature to remove residual organic solvent. The film was mixed with water (400 μL) and sonicated 3 times for 15 seconds, diluted with 500 μL of water, and sonicated again two times for 30 seconds. Molar ratios of PFPE amide 1 to DMPC were 1:2, 1:1.5, 1:1, 1:0.5 and 1:0.25, as shown in Figure 9. All emulsions were clear and they were kept at room temperature for 24 hours before DLS measurements. As the amount of lipid was decreased, the particle size increased and the polydispersity decreased. An optimal particle size (158.5±1.0 nm) and PDI (0.17-0.19) was achieved at a molar ratio 1:0.5 PFPE amide 1 to DMPC, as is shown in Figure 10.

To test the effect of high-energy emulsification on the PFPE amide 1/lipid mixtures, the same procedure as above was used, and all emulsions were split in half. One half was sonicated and the other half was not. The particle size decreased significantly when sonication was used, as shown in Figure 11.

Introducing a neutral lipid did not change the intrinsically negative zeta potential of the PFPE amide 1 emulsified in water. The zeta potential was also negative when PFPE amide 1 was emulsified with neutral Pluronics™ in water or 1X H BSS. The neutral lipid DMPC was used as a model to test feasibility of mixing PFPE amide 1 with lipids. Cationic lipids (CTAB or DDAB) were then introduced to investigate reversal of the

negative zeta potential of PFPE amide 1 particles made with Pluronics™, as shown in Figure 14.

DMPC/DDAB/PFPE amide 1:

In a round bottom glass tube, DMPC (18 mg), DDAB (0-8 mg) and PFPE amide 1 (76 mg) were dissolved in trifluoroethanol (400 µL), vortexed and dried into a thin film using a stream of argon gas. The film was then further dried for 15 minutes under vacuum. Water (1 mL) was added while vortexing vigorously, and the emulsion was heated for 15 minutes at 60 °C. Each emulsion was divided in two 0.5 mL portions, and one was sonicated for 30 seconds at room temperature, and the other was left untreated at room temperature. All samples were diluted by 1/3 with water prior to DLS measurements. The DLS measurements showed a clear need for sonication in this system to decrease particle size and polydispersity. Brief sonication (< 20 sec) gave a dramatic reduction in particle size and polydispersity. The particle size was the smallest with 4% DDAB, as shown in Figure 12. When introducing lipids to PFPE emulsions, due to the high lipophobicity of PFPE, high energy emulsification methods were needed. Lipophobicity of PFPE also explains why stability of lipid-containing emulsions was lower then in emulsions made with Pluronics™ alone.

CTAB/PFPE amide 1:

CTAB and PFPE-amide 1 were dissolved in trifluoroethanol (300 µL) in a round bottom glass tube. Argon gas was blown into the tube through a pipette tip until a homogenous off-white film was formed. The tube was connected to vacuum for at least 30 minutes. Water medium or 1X HBSS were added (1 mL/sample) while vortexing vigorously and then the tube was placed in a water bath at 60 °C for 15 minutes. Each sample was then sonicated for 30 sec to homogenize the particles. The DLS measurements showed no change in particle size with increasing amounts of CTAB. There was also no change in size when L35 was used as co-emulsifier, as shown in Figure 13. However, the zeta potential did increase with CTAB addition, as shown in Figure 14. The percentages shown are with respect to the dry material weight.

PFPE amide 1/Polyethylamine emulsion by sonication:

Polyethylenimine (PEI) is a polyamine transfection agent known to help DNA, RNA and proteins enter into cells. Low molecular weight PEI (e.g., 430 or 800) was utilized as both a coemulsifier and a means to introduce primary amino groups to facilitate cellular uptake of the nanoparticles. The goal was to introduce a sufficient number of free amino groups and to keep the particle size and PDI as low as possible. PEI also serves as a surfactant due to large number of hydrophilic groups (i.e., primary amino groups).

The primary amines in polyethylamine (PEI, average MW = 800) interact with highly reactive PFPE methyl ester 39 end groups. 5% of PFPE methyl ester 39 mixed with PFPE amide 1 was used for emulsification with PEI. PEI was dissolved in absolute ethanol (100 mg/mL). PFPE methyl ester 39/PFPE amide 1, 5:95 v:v (50 µL), was vortexed with water (450 µL) and PEI ethanol solution (100 µL), placed on a rotovap at 40 °C to remove ethanol under vacuum, and then sonicated two-times for 10 sec. The solution was diluted four-times to measure particle size by DLS, as shown in Figure 15.
This was the first PFPE/PEI emulsion ever prepared and PFPE was never before combined with polyethylenimines. Highly hydrophilic PEI serves as an emulsifier and gives needed amino groups for uptake.

PFPE amide 1/Protamine sulfate nanoparticles by sonication:

Protamine sulfate is a common transfection reagent, and it is highly biocompatible. Protamine sulfate is FDA approved for human use and clinically used as an antagonist to heparine. Its low toxicity makes it a desirable delivery reagent for DNA and RNA. Nanoparticles of PFPE amide 1 formulated with protamine sulfate were designed to promote cellular uptake. For each test emulsion of PFPE amide 1 with protamine sulfate, PFPE amide 1 (50 µL) was sonicated for 10 sec in 300 µL of deionized water. Protamine sulfate (salmine sulfate, USP) solution in water (300 µL, 0 to 20mg/mL) was added and vortexed vigorously for 20 sec. The emulsions were sonicated for 10 sec and diluted 1/3 in water for DLS measurements. Increasing amounts (% w/w) of protamine sulfate led to an increase in particle size and zeta potential, as shown in Figures 16 and 17.

Protamine sulfate coated PFPE amide 1/L35 emulsion:

PFPE amide 1 was emulsified with L35 using sonication, in molar ratio 1:1, as described above, and diluted in serum free media containing low concentration of

protamine sulfate (10μg/mL). The mixture was incubated at room temperature for 30 min. The emulsion was analyzed by DLS without further dilution, as shown in Figure 18. Coating PFPE amide 1/L35 emulsion droplets with protamine sulfate increased the droplet size and PDI by 30%. This increase in size had no detrimental effects on cellular uptake of PFPE/L35 particles, as discussed below. Coating the PFPE amide 1/L35 nanoemulsion droplets with protamine sulfate increased uptake in jurkat cells several fold (Figure 35B) without having any negative effects on the cell viability (Figure 35A).

These initial experiments with incorporating protamine sulfate into the PFPE nanoemulsions served as the basis for the design of highly stable and very efficient PFPE/F68/Protamine Sulfate emulsions. In this new emulsion the protamine sulfate was incorporated into the nanoparticles at low amount (0.01-1% w/w) by microfluidization.

Microfluidization emulsification examples:

Sonication methods are effective on small scale, resulting in small size particles (< 200 nm) and low PDI (0.1-0.3) in most cases. However this method cannot be used for large scale preparations. Our goal was to prepare batches of stable emulsions for repeated cell labeling and MRI imaging. Presented studies indicate a clear need for high energy methods. Microfluidization utilizes high shear forces to decrease the droplet size of the prepared emulsions and exhibited a decreased PDI.

Microfluidization of PFPE amide 1/F68:

Pluronic™ F68 is a nontoxic and FDA approved emulsifier. It has been approved in presence of egg yolk lecithin for emulsification of perfluorocarbons in blood substitutes preparations. F68 has a high molecular weight (8400) and provides greater stability to PFPE nanoemulsions compared to L35. F68 is used as an emulsifier for most of the examples presented below using microfluidization, and in some cases in combination with sonication.

PFPE amide 1 (1mL) was mixed by vortexing with F68 solution in 1X HBSS buffer (67mg/mL, 4mL), followed by three periods of sonication each lasting for one minute, where the mix was cooled on ice between the periods for 30 sec. The emulsion

was then diluted to 20 mL with 1X HBSS and passed through a microfluidizer (M-110S Microfluidics, Inc. Newton, MA) at 40 psi working air pressure. Initial sonication produced large particles (~ 345 nm) and large PDI (0.4) due to low energy transfer efficiency from the tip of sonicator through the large volume (4 mL) of the pre-emulsion. The first pass through the microfluidizer had the most dramatic effect, decreasing particle size by 100 nm, and decreasing the PDI three-fold. Further passages did not have a significant effect on particle size, and PDI stayed constant, as is shown in Figure 19. PFPE amide 1/F68 emulsion was monitored by DLS for one week. It showed no changes in particle size and PDI over this period, as is shown in Figure 20. Particle size of PFPE amide 1/F68 microfluidized emulsion was confirmed by electron microscopy (EM), as is shown in Figure 21.

Microfluidization of PFPE amide 1/L35 and PFPE amide 1/L64:

PFPE amide 1 (637μL), L35 (9μL) or L64 (13.8 μL) were mixed by vortexing with 13.2 mL of 1XHBSS and passed through a microfluidizer 2 times at 40 psi working pressure. The emulsions were vortexed again, sealed in containers and stored at room temperature for four days prior the first DLS measurements.

The emulsion sedimented slowly but homogenized quickly by simple shaking. No oil separation was observed during the follow-up. Particle size and PDI stayed constant over six weeks of testing, as is shown in Figure 22. When the PFPE amide 1 without Pluronics™ was microfluidized, the emulsion had the same particle size, but it degraded and oil drops formed in only two days. It was clear that a small amount of Pluronics™ was necessary to keep the droplets of PFPE amide 1 from coalescing. The molar ratio of PFPE amide 1 to Pluronics™ was 100:1.

Microfluidization of PFPE amide 1/F68 with PEI

Polyethylenimine (PEI, MW~430) was not coupled directly to PFPE, but incorporated into the emulsion during processing to coat the particles with primary and secondary amine groups that are known to promote cellular uptake. PFPE diethyl amide 1 (10% w/w) was emulsified with F68 in water by microfluidization. PFPE-diethyl amide 1 (1.52g, 1mL) and F68 solution (0.68 mL, 100mg/mL in water) were mixed, vortexed in water (6.7mL) and then processed using an Ultraturax device (Tekmar, Cincinnati, OH) for

5 min at room temperature. PEI (Mn~430) solution (0.52 mL, 100mg/mL in water) and 6.7 mL of water were added, and the emulsion was vortexed on high for 2 min. The pre-emulsion was passed through the microfluidizer while the processing chamber was chilled on ice. The processing included two sequential passes at a 50 psi working pressure. A control emulsion (PFPE-diethyl amide/F68) was prepared the same way without the PEI solution. Particle size was 200 nm on average and the PDI ranged form 0.1-0.2. The presence of PEI did not affect the particle size. Stability was tested at 4, 25 and 37°C for three weeks using DLS measurements. The PFPE/F68 emulsion was stable at 37°C for at least two weeks and the PFPE/F68/PEI emulsion was stable for at least three weeks (Figure 23); these data represent sequential measurements of particle size at different temperatures. The emulsions were stored at the original concentration and diluted 10-fold prior to each DLS measurement. No significant changes in PDI were observed. These two emulsions were compared in uptake experiments using Jurkat cells, and a significant increase in uptake was observed when PEI was present, as shown in Figure 41.

PFPE-oxide /F68/PEI

PFPE-oxide (1a) is an analogue of PFPE that has no hydrocarbon end groups and has lower average molecular weight (MW~1300). PFPE-oxide is a mixture of polymers custom synthesized by direct fluorination of PEG-OH (Exfluor, Roundrock, TX), and its main peak at -91.6ppm corresponds to 36 fluorine spins. PFPE-oxide emulsifies similarly to PFPE-diethyl amide using microfluidization with F68, as shown in Figure 24A. Therefore, PFPE-oxide can replace PFPE diethyl amide in cell labeling applications where further conjugation of PFPE to targeting agents or fluorescent dyes is not required. Furthermore, PFPE-oxide 1a has very similar physical properties to PFPE amide 1, and was used blended with PFPE amide 1 and FBPA (fluorescent blended PFPE amides) in the preparation of novel blended PFPE oil nanoemulsions. The PFPE blending capabilities opened new avenue for nanoemulsion preparations and resulted in highly stable fluorescent and non fluorescent PFPE nanoemulsions.

PFPE-oxide (1.7g, 1mL), F68 (68mg) and PEI (52mg) were emulsified in water (14mL) by passage through a microfluidizer twice under 50 psi pressure. A second emulsion was prepared in the using 1 mL of PFPE-diethyl amide, and both emulsions were

compared by DLS measurements. Particle size, PDI and counts were virtually identical (Figure 24A). The PFPE-oxide/F68/PEI emulsion showed excellent stability. It was monitored over a 250 days period by DLS to evaluate its stability at three different temperatures, 4, 25 and 37 ^{o}C. The PFPE-oxide emulsion showed no change in particle size at all three temperatures over this time course, as shown in Figure 24B. The PFPE-oxide/F68/PEI is a highly stable emulsion with a very low PDI (<0.1). This emulsion gave a reproducible, dose-dependent uptake in Jurkat cells, as shown in Figure 42.

The ^{19}F NMR spectra of the PFPE diethyl amide and the PFPE-oxide emulsions were compared (Figure 25). The main PFPE peaks of these two materials are at the same chemical shift (-91.0 ppm), and thus the PFPE oxide 1a can be used for 19F MRI imaging interchangeably with the PFPE-diethyl amide.

Nanoemulsions with Protamine Sulfate by Microfluidization

PFPE amide 1/F68/protamine sulfate:

PEI provides much improved uptake in cells and can be incorporated directly into the nanoemulsion during the emulsification process by either sonication or microfluidization. However this polyamine is not FDA approved for human applications. Therefore, Protamine sulfate was introduced in the preparation of highly stable nanoemulsions by microfluidization. Protamine sulfate is added to the surfactant (F68) and PFPE mixture in the pre-emulsion as an aqueous solution, and incorporated into the nanoemulsion droplets during microfluidization processing. The ratio of protamine sulfate to PFPE can vary from 0.01%-1% w/w. This amount of PFPE provides significant uptake increase without changing the sign of zeta potential to positive.

PFPE amide and PFPE oxide (1a) were emulsified by microfluidization with protamine sulfate. The goal was to prepare protamine coated particles during the processing and avoid the extra step of coating the emulsion particles with this material prior to its use for cell labeling. PFPE amide 1 (3.4 g, 2 mL) was mixed by vortexing with F68 (1.36 mL, 100mg/mL in water) and protamine sulfate (0.5 mL, 20 mg/mL in water, 0.06% w/w to PFPE). Water was added (5 mL) and the vortexing was repeated for 1 min. The remaining water (15 mL) was added and the emulsion was vortexed again for 30 sec prior to microfluidization. The emulsion was microfluidized using 20- 40 pulses at 80-100

psi working air pressure (corresponding to 12000-16000 psi liquid pressure) while the processing chamber was chilled on ice. Each pulse accounts for one pass through a processing chamber.

PFPE oxide (1a)/F68 with/without protamine sulfate:

Two PFPE-oxide emulsions were prepared, with and without protamine sulfate. The emulsions were prepared in a similar manner as described above for PFPE amide. PFPE oxide 1a (4mL) was added to a 50mL tube along with F68 solution (2.72mL) and the mixture vortexed on highest speed for 1 minute. Protamine Sulfate solution (1mL) was then added and the mixture vortexed again on highest speed for 1 minute. If no protamine sulfate was added, 1mL of deionized water was added at this step. Next, 12mL of water was added and the solution was vortexed again on highest speed for 1 minute. The emulsion was then transferred into the sampling chamber of the Microfluidizer, pressed with the plunger, and the plunger was held tight while pulsing exactly 40 times at the air pressure of 80-90psi. At pulse number 40 the product was released. At this small scale there was dead volume in the lines and processing chamber. 5mL of water was added and the left over product was slowly flushed out. The volume was adjusted with 25mL water at the end if necessary. This method was used for the 100mL scale, where four 25mL batches were combined at the end. The batches were filtered immediately through Syringe Filter with a 0.2um PTFE membrane from Pall Life Sciences without pretreatment. The shelf life for PFPE-oxide/F68 nanoemulsion and PFPE-oxide/F68/Protamine Sulfate nanoemulsion is shown in Figure 54. Data represents the diameter and polydispersity measured by DLS at three different temperatures, as previously described, over 250 days. Both emulsions remained stable at 4°C. The PFPE-oxide/F68 emulsion began to break down at higher temperature much sooner then PFPE-oxide/F68/Protamine Sulfate, Figures 54A and 54B respectively.

PFPE oxide 1a emulsion with protamine sulfate showed a significant increase in uptake in DCs as compared to the PFPE oxide 1a/F68 emulsion without protamine sulfate, Figure 55.

Serum stability testing:

All large scale preparations of emulsions for *in vivo* work should ideally undergo stability tests in presence of nutrients, culture media and relevant serum content. These tests are crucial for the development of non-toxic and stable emulsions that give reproducible and reliable cell labeling in vitro. Emulsions were tested by DLS measurements in the presence of serum and cellular media nutrients at 37 °C. Emulsions were mixed with cell culture media containing 10% FBS at the cell labeling concentration and incubated at 37°C for 1, 3 and 24 h. After each time point emulsions were tested by DLS. In presence of serum, PFPE oxide 1a emulsion was far more stable then PFPE amide 1 emulsion even though the particle size and PDI were initially the same, as shown in Figure 26.

Nanoemulsions from "blended" PFPE oils by microfluidization at high processing pressure

All the emulsion preparations described below use two or three PFPE derivatives (PFPE amides, PEG PFPE amides or FBPAs), blended further with PFPE oxide 1a. The addition of PFPE oxide 1a achieves better serum and in vivo stability. This all relies on the fact that PFPE mixes best with itself and forms its own fluorocarbon oil phase. It is also important that PFPEs are close in specific gravity, which range from 1.5-1.7 g/mL.

Blended PFPE oil emulsification was tested first by blending PFPE amide 1 and PFPE oxide 1a into one fluorinated oil phase that was then used for nanoemulsion preparation by microfluidization.

The FBPAs with FITC, BODIPy-TR and Alexa647 dyes, described above, were also blended with PFPE oxide 1a and used for fluorescent nanoemulsion preparation by microfluidization.

PFPE oils, FBPA or PFPE amide and PFPE oxide 1a were first blended together by continuous vortexing on the highest speed for 5 minutes, followed by mixing with F68 and PEI (or protamine sulfate) water solutions and then microfluidized. High mechanical energy applied within the interaction chamber of the microfluidizer disperses PFPE hydrophobic oil in water and results in monodisperse nanoemulsions. Non-fluorescent PFPE nanoemulsions were prepared by mixing PFPE oxide 1a and PFPE amide 1 at a ratio 9/1 v/v. Fluorescent nanoemulsions were prepared from PFPE oxide 1a and FBPA at 9/1 v/v ratio. All nanoemulsions were filter-sterilized (PTFE filter, pore size 0.22 μm) and stored at 4 °C until use for cell labeling. A Microfluidizer® M110S (Microfluidics, Inc.,

Newton, MA) operating at a liquid pressure of approximately 15000-20000 psi (80-100 psi working air pressure) was used for "blended" PFPE oils nanoemulsion preparations. PFPE oils (FBPA or PFPE amide 1 and PFPE oxide 1a) were mixed first with a concentrated solution of F68 in water (100 mg/mL) by vortexing on high speed for 2 minutes and then mixed with polyethylenimine (PEI) solution in water (100 mg/mL) by vortexing for 2 minutes. The mixture was diluted to 25 mL with water followed by microfluidization. After 20-40 pulses, the final nanoemulsion product was drained into a collection container and sat at r.t. for 20 minutes, followed by sterilization using a PTFE (0.2 μm) filter. The emulsion was stored at 4 °C until use.

Fluorescent blended PFPE nanoemulsion BODIPy-TR PFPE amide/PFPE oxide 1a/F68/PEI was used to label primary T cells. Figure 56 shows confocal microscopy of labeled T cells. FITC PFPE amide/PFPE oxide 1a/F68/PEI nanoemulsion was also used for cell labeling. Figure 58 shows labeled DCs where green fluorescence indicates cytoplasmic localization of the FITC PFPE nanoemulsion. In addition, BODIPy-TR PFPE labeled jurkat cells demonstrate linear correlation between fluorescence and 19F NMR signal, Figure 57.

BODIPy-TR PFPE nanoemulsion preparation:

BODIPy-TR PFPE amide oil (0.20 mL) and PFPE oxide 1a (1.80 mL) were mixed first by vortexing on high for 5 minutes to achieve a final concentration of BODIPy-TR dye in the blended PFPE oil of 0.46 mM. The fluorescently labeled PFPE oil was then mixed with F68 solution (1.36 mL, 100 mg/mL) in water and vortexed for 2 minutes. To this mixture PEI solution (1.04 mL, 100 mg/mL) in water was added, and vortexing was repeated for 2 minutes at the highest speed. Water was added to a final volume of 25 mL; after brief vortexing, the mixture was loaded into the sample chamber of the microfluidizer. The final emulsion was drained after 20 pulses. The prepared nanoemulsion was stored at 4 °C until use.

Alexa647 PFPE and FITC PFPE nanoemulsions preparations:

Following the same procedure Alexa647 PFPE amide and FITC PFPE amide were used for fluorescent nanoemulsion preparations. In each case the same volume of FBPA (0.2mL)

was mixed by vortexing with PFPE oxide 1a (1.8mL) while the rest of processing was done the same way as for BODIPy-TR PFPE amide, described above.

PFPE amide 1/PFPE oxide 1a nanoemulsion preparations:

Following the same procedure as above PFPE amide 1 and PFPE oxide 1a were blended together by vortexing and then used for emulsion preparation by microfluidization.

All "blended" PFPE oil nanoemulsions, fluorescent and non fluorescent were stability tested at three temperatures (4, 25 and 37 °C) by monitoring nanoemulsion droplet diameter (Z average) and polydispersity (PDI) over time by dynamic light scattering (DLS) measurements. The particle size was lowest for PFPE amide 1/PFPE oxide 1a blended nanoemulsion, only 140nm, while the fluorescent versions regardless of the dye (FITC, BODIPy-TR or Alexa647) showed the average droplet size 160-190nm. All emulsions were successfully sterile filtered and remained stable for at least 3 months at 37 °C and at least 6 months at 4 and 25 °C. Serum stability was unaffected by the presence of PFPE amide 1 and FBPA in the blended PFPE oils used for these emulsion preparations and virtually the same as serum stability of emulsions prepared with PFPE oxide 1a alone.

Blended PFPE oils nanoemulsions prepared by microfluidization with linear PFPE oils and perfluoro-15-crown 5 ether blended together

As described above, blending PFPE oils has many advantages. PFPE amide 1, FBPA and PFPE oxide 1a readily blend with each other and behave as a discreet fluorinated oil phase in nanoemulsion preparations. These emulsions are highly stable and have low average droplet size and PDI. Perfluoro-15-crown 5 ether is a highly chemically stable and biologically inert macrocycle with 20 equivalent fluorine nuclei giving single resonance that overlaps with the CF_2CF_2O repeat resonance of the linear PFPE main peak, at -91.5 to -92.0 ppm. This clear oil with quite similar density to linear PFPE oxide 1a, slightly lower viscosity. Nanoemulsions that are made by blending linear PFPE derivatives (e.g., FBPAs and PFPE amides) with perfluoro-15-crown 5 ether, which cannot be further chemically modified and conjugated, were investigated. This approach provides access to fluorescent

perfluoro-15-crown 5 ether emulsions. In certain embodiments, the majority of the nanoemulsion droplet comprises crown ether as compared to linear PFPE derivative (e.g., the nanoemulsion droplet comprises 75%-95% crown ether as compared to linear PFPE derivative).

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General procedure for blended linear PFPE and crown ether nanoemulsions:

Linear PFPE oils, FBPA or PFPE amide or PFPE oxide 1a are first blended together with perfluoro-15-crown-5 ether by continuous vortexing on the highest speed for 5 minutes, 10 followed by mixing with F68 and PEI (or protamine sulfate) water solutions and then microfluidized. Due to high hydrophobicity and lipophobicity the linear and macrocyclic PFPE stay together as one fluorocarbon phase. The ratio of linear to crown PFPE is (99:1 to 75:25). The linear PFPE introduces functionality (fluorescence dye) to PFPE crown ether in this blended oil that would otherwise be impossible. The same approach can be 15 used to introduce fluorescent dyes to perfluoro-15-crown-5 ether nanoemulsions prepared with lipids, cremaphor oils or other types of ionic and non-ionic surfactants.
The preparation of blended oils is easily scalable following the same methodology as described above for FBPA emulsions.

20 **Example of nanoemulsion prepared with blended PFPE amide 1 and Perfluoro-15-crown ether.** PFPE amide 1 oil (0.20 mL, 1.25% w/w) and Perfluoro-15-crown-5 ether (1.80 mL, 12.75% w/w) were mixed first by vortexing on high for 5 minutes to achieve homogenous blending of two oils. The blended oil was then mixed with F68 solution (1.36 mL, 100 mg/mL) in water and vortexed for 2 minutes. To this mixture Protamine Sulfate 25 solution in water (0.53 mL, 18.75 mg/mL) was added, and vortexing was repeated for 2 minutes at the highest speed. Water was added to a final volume of 25 mL; after brief vortexing, the mixture was loaded into the sample chamber of the microfluidizer. The emulsion was microfluidized at 80 psi working air pressure. The final emulsion was drained after 30 pulses. The prepared nanoemulsion was sterilized by filtration through a 30 nylon filter and stored at 4 ^{o}C until use. The particle size for this emulsion was 160nm. The emulsion remained stable at 37 ^{o}C for 3h in presence of serum containing media. The

uptake in non-phagocitic cells of this emulsion was the same as for PFPE oxide 1a/F68/Protamine Sulfate nanoemulsion reported earlier in this text.

Low energy emulsification examples:

Previous examples showed the need for high energy when PFPE amide 1 or PFPE oxide (1a) is emulsified in aqueous media in presence of lipids or Pluronics™. In these examples we show that simple PFPE end group modifications allow for PFPE emulsification without sonication or microfluidization. The goal was to develop PFPE derivatives that promote self-emulsification in the presence or absence of emulsifiers, by introducing either lipophilic or hydrophilic moieties onto the PFPE end groups. Low energy emulsification methods can be easily utilized for larger scale production and decrease the emulsification processing cost. Two such PFPE derivatives are shown bellow.

PFPE-tyramide 6/ F68 emulsion by a thin film method:

PFPE-tyramide 6 (50 mg) and F68 (8.5 mg) were dissolved in trifluoroethanol (0.5 mL) and vortexed. The solution was dried into a thin film in a round bottom glass tube and placed on vacuum for 15 minutes. The film was vortexed with 1 mL of water and heated at 60 °C for 15 minutes, then cooled to room temperature while vortexing on high. Serial dilutions were made ranging form 1/1 to 1/32 in water and DLS were measurements performed. The critical micell concentration (CMC) was estimated to be 4.5 mg for PFPE tyramide 6 in this system, by plotting the light scattering intensity and particle size as a function of concentration of PFPE tyramide 6 (Figure 27). The mole ratio of PFPE-tyramide 6/F68 was 24:1. This emulsion was monitored by DLS for three weeks to test stability. The emulsion was kept at room temperature and protected from light and air. The particle size and PDI stayed constant over the three week period, as shown in Figure 28.

PFPE (2-hydroxyl)ethyloxyethyl amide 2/ F68 emulsion:

PFPE (2-hydroxyl)ethyloxyethyl amide 2 was investigated as an alternative to the PFPE-tyramide derivative. The presence of 2-hydroxyl)ethyloxyethyl amide end group was to promote PFPE self-emulsification under low energy conditions. It was found that this derivative emulsifies readily in water in presence of Pluronics™ at low temperature.

PFPE amide derivative 2 (50 mg) and F68 (8.5 mg) were dissolved in trifluoroethanol and dried to a thin film by a stream of argon gas. Water or 1X HBSS (1mL) was added while vortexing. The emulsion was then split in two parts, where one part was incubated at 60 °C for 15 minutes and the other part was left at room temperature. The effects of buffer and temperature on emulsification with F68 were investigated by DLS measurements. The PFPE amide derivative 2 could not form an emulsion without F68 by the applied methods. Temperature had no effect on particle size or PDI, as shown in Figure 29. However, the presence of salts in the 1X HBSS buffer increased the particle size. This example illustrates that the structure and composition effects the emulsification properties of PFPE by low energy emulsification methods. Factors that affect PFPE emulsification include, for example, the nature of the PFPE end groups (i.e., lipophilic or hydrophilic), the emulsifier, the external phase, and the presence of salts in the buffer.

Overall, simple and effective chemical modifications were developed to provide multifaceted MRI/MRS cell labeling reagents. PFPE is shown to be a versatile starting material for a variety of nanoparticles, including dual fluorescent-19F MRI/MRS reagents, self delivering PFPE nanoparticles, and nanoparticles with highly efficient uptake in both phagocytic and non-phagocytic cell types. PFPE-PEG derivatives can be used for direct injection experiments, where cellular uptake is not desired and prolonged circulation stability is necessary. PFPE-PEI and PFPE-protamine sulfate and the like can be used for efficient cellular labeling. Microfluidization is a very effective method for PFPE nanoparticle preparations, allowing for large scale production (>1L) of emulsions. The emulsions produced were highly stable at typical storage temperatures (4 and 25 °C) and body temperature (37 °C). Simple changes in the emulsification process and simple chemical modifications of PFPE end groups allow fine tuning of nanoparticle properties towards specific cell types and applications.

Example of nanoemulsion prepared with blended FBPA (FITC or BODIPy-TR PFPE amide) and Perfluoro-15-crown ether:

FBPA (FITC PFPE amide or BODIPy-TR PFPE amide) oil (0.20 mL, 1.25% w/w) and Perfluoro-15-crown-5 ether (1.80 mL, 12.75% w/w) were mixed first by vortexing on high for 5 minutes to achieve homogenous blending of the two oils. The blended oil was then mixed with F68 solution (1.36 mL, 100 mg/mL) in water and vortexed for 2 minutes. To this mixture Protamine Sulfate solution in water (0.53 mL, 18.75 mg/mL) was added,

and vortexing was repeated for 2 minutes at the highest speed. Water was added to a final volume of 25 mL; after brief vortexing, the mixture was loaded into the sample chamber of the microfluidizer. The emulsion was microfluidized at 80 psi working air pressure. The final emulsion was drained after 30 pulses. The prepared nanoemulsion was sterilized by filtration through a nylon filter and stored at 4 °C until use. The particle size for this emulsion was 165nm and PDI 0.06. The emulsion remained stable at 37 °C for 3h in the presence of serum containing media. The emulsion remained stable at 37 °C storage temperature for 5 months, without change in size or PDI. Labeling efficiency of non-phagocitic cells (9L, rat glioma cell line) was the same as for PFPE oxide 1a/F68/Protamine Sulfate nanoemulsion above. Labeling of non-phagocytic 9L cells is shown in figure 60.

Formulations prepared with mixed micelle and PFPE by microfluidization

The following emulsions were designed to specifically label macrophages and other phagocytic immune cells both *in vitro* and *in vivo*. The enriched PEG containing nanoemulsion droplet surface prevented the nanoemulsion uptake in cells lacking phagocytic ability. The PEG enriched surface was introduced by two surfactants, well known in the art, pluronic P105 and Cremophor EL. The mixed micelle solution was prepared with P105 and Cremophor EL first, and PFPE blended oils incorporated into the micelle by high shear forces during microfluidization. The resulting emulsions showed high stability and very specific labeling activity towards RAW 264.1 macrophage cell line *in vitro*.

Example of nanoemulsion prepared with blended FBPA (BODIPy-TR PFPE amide) or PFPE amide 1 and Perfluoro-15-crown ether with PEG enriched surface:

Mixed micelle solution was prepared first in sterile saline solution with P105 pluronic and Cremophor EL at 2/3 w/w ratio. Pluronic P105 (4g) was dissolved in 100 mL of normal sterile saline solution (0.9% NaCl) by stirring slowly at room temperature to a final concentration of 4% w/v. Cremophor EL (6g) was dissolved in 100 mL of normal sterile saline solution by stirring at room temperature to a final concentration of 6% w/v. The two solutions were mixed at room temperature in 1/1 v/v ratio in a round bottom flask, placed in a water batch preheated to 45 °C, and incubated while slowly rotating for 15

minutes. The solution was then chilled on ice for 5-10 minutes. The prepared mixed micelle solution contained micelle particles with average size of 12.3 nm and PDI 0.17. This mixed micelle solution was used for preparing PFPE nanoemulsions as follows.

PFPE amide 1 or FBPA (BODIPy-TR PFPE amide) oil (0.20 mL, 1.25% w/w) and Perfluoro-15-crown-5 ether (1.80 mL, 12.75% w/w) were mixed first by vortexing on high for 5 minutes to achieve homogenous blending of two oils. The blended oil was then mixed with 11.5 mL of mixed micelle solution by vortexing on high speed for 2-3 minutes. Saline solution was added (11.5 mL); after brief vortexing, the mixture was loaded into the sample chamber of the microfluidizer. The emulsion was microfluidized at 80 psi working air pressure while the processing chamber was chilled on ice/water bath. The final emulsion was drained after 20 pulses and stored at 4 ^{o}C until use. The average droplet size was 130-150 nm and PDI 0.05-0.1. The emulsions were stable at 37 ^{o}C for up to 24h in presence of serum containing media. Shelf life stability tests showed the size and PDI remain unchanged for 3 months at 4 ^{o}C storage temperature. The emulsions were used to label RAW 264.1 cells (rat macrophage derived cell line) in vitro by 24h co-incubation with cell viability at 80% or higher. The dose dependent labeling of RAW 264.1 cells with BODIPy-TR PFPE amide P105/Cremophor EL emulsion is shown in Figure 61. Fluorine NMR spectrum of labeled RAW cells is shown in figure 62.

The two presented examples of PFPE/P105/Cremophor EL emulsions did not label non-phagocitic cells, and specifically labeled macrophages (data not shown). This was due to high density of PEG monomers on the particle surface that sterically hindered the cell membrane and nanoemulsion droplet interaction. Due to this steric hinderence, the cell labeling of macrophages takes 24 h, which is longer then in earlier presented examples.

Example of nanoemulsion prepared with Compound **42**:

Mixed micelle solution was prepared as follows: Pluronic P105 (5g) was dissolved in 100 mL water by stirring slowly at room temperature for a final concentration of 5 % w/v. Cremophor EL (5g) was dissolved in 100 mL water by stirring at room temperature for a final concentration of 5 % w/v. The two solutions were mixed at room temperature in 1/1 v/v ratio in a round bottom flask, placed in a water bath preheated to 45 ^{o}C, and incubated while slowly rotating for 20 minutes. The solution was then chilled on ice for 5-15 minutes, and stored at room temperature until use. The final concentration of mixed

micelle solution was 5% w/v, where 2.5% w/v was P105 and 2.5% w/v was Cremophor EL, present in 1/1 w/w ratio. The mixed micelle solution was used for compound **42** nanoemulsion preparations by sonication.

All nanoemulsions were prepared by sonication during which compound 42 was loaded into the core of mixed micelle. The amount of surfactants necessary for optimal cell labeling was optimized. A series of nanoemulsions were prepared as shown in Table 1. Briefly, compound 42, which is a clear colorless oil heavier then water, was added to the sonication tube first, followed by varied amounts of micelle solution and water, vortexed briefly and then sonicated at room temperature. The amount of fluorocarbon was kept constant at 22.3%, while the amounts of Cremophor EL and pluronic P105 were varied from 4% w/v, as in emulsion A1, to 1% in emulsion A4. As shown in Table 1, as the amount of surfactant increased the droplet size of the nanoemuslions decreased.

Table 1. Nanoemulsion compositions prepared with mixed micelle solution and compound 42

Nano-emulsion	P105/ Cremophor EL Mixed micelle Solution (μL)	Cremophor EL and P105 (% w/v)	Comp. 42 (μL)	Water (μL)	Size (nm)	PDI	Zeta Potential (mV)
A1	400	4	100	0	131.1	0.13	-3.15
A2	300	3	100	100	145.3	0.12	-2.23
A3	200	2	100	200	160.2	0.14	-3.82
A4	100	1	100	300	182.5	0.17	-3.33
	500	0	0	0	14.1	0.11	-6.65

3. Cell labeling experiments with fluorescent and non-fluorescent PFPE nanoemulsions

Cell labeling using the nanoemulsions was demonstrated in both phagocytic cells, using a fetal-skin derived mouse DC line, and in non-phagocytic cells, including primary T cells and Jurkat cells. In all cell types studied, suitable levels of emulsion uptake for *in vivo* MRI (i.e., $>10^{11}$) were achieved in a modest 3 hour incubation period. Intracellular localization of the nanoparticles was visualized using fluorescence microscopy. Cellular

uptake of nanoemulsion droplets was quantified using fluorescence measurements and by ^{19}F NMR spectroscopy of lysed cell pellets. Uptake was tested by ^{19}F NMR, where the PFPE labeled cells show a major peak at -91.58 ppm; the -76.00 ppm peak is from TFA reference added to lysed cell pellet. The integrated areas under these two peaks can be used to calculate the mean ^{19}F/cell (see Experimental), often ranging from 10^{11}-10^{13}. The same approach was used for evaluating uptake in all cell types tested.

Cell number and viability:

Cell viability after PFPE labeling may depend on a variety of factors, for example these may include: the PFPE derivative used, the surfactants used for emulsion preparation, the presence of lipids, the particle size, the serum stability of the emulsion and the washing efficiency. In vitro assays for cell number and viability of fluorocarbon-labeled cells are widely known in the art.

In the examples given below, cellular number was estimated using the commercial assay Cell Titer Glo (Promega). The assay implementation is easy and highly reproducible. A correlation curve was constructed for each labeling experiment at the day of experiment using the same cell culture. When testing the viability post- labeling, a small sample (50 µL) was taken from labeled cell suspension and mixed with Cell Titer Glo reagent in triplicate. Luminescence was measured within 10 minutes and the viable cell number was estimated from a linear correlation curve prepared on the day of experiment.

Toxicity and serum instability are often key screening criteria for newly developed PFPE formulations, in addition to good DLS profiles (i.e., small particle size and low PDI). The PFPE/CTAB/L35 emulsion was an example where high toxicity stopped further development. The toxicity came from CTAB primarily, even at the lowest dose, where cells detached post-labeling within one hour. The emulsion dose that was safe was too low to provide useful labeling.

PFPE/L35 and PFPE/F68 combinations were examples of useful formulations for cell labeling. Pluronics™ L35 and F68 were non-toxic when administered to cells alone. Various PFPE derivatives were tested for efficiency of cell labeling with L35 or F68 Pluronics™ with and without polyamines, PEI or protamine sulfate.

PFPE labeled cells were tested for long term viability *in vitro* by replating labeled cells in 96 well plates at the same density as untreated controls and following them over

five days by testing their viability by Cell Titer Glo each day. Growth curves were constructed. Labeled and non-labeled cells had the same growth profile, confirming that the PFPE label was non-toxic. Figure 37 shows growth of Jurkat cells labeled with several different PFPE formulations measured by Cell Titer Glo and performed in triplicate.

3.1. PFPE Labeling of Attached Cells

Labeling mouse dendritic cells was performed according to published methods with minor modifications[35]. Dendritic cells were plated in 6-well plates, 1-2x10^6/well, and allowed to attach overnight. A PFPE diethyl amide 1/L35 emulsion was mixed with 0.3 mL of serum free media, incubated for 30 min at room temperature, and then added to the cells. After a 3 h incubation at 37 °C, the cell labeling medium was removed and cells were washed three times with 1X PBS, detached by trypsinization, washed, and resuspended in 0.5 mL of complete media containing 10% FBS. A portion of the cell suspension (1/10) was used for cell number estimates by Cell Titer Glo. The cells were pelleted\ and resuspended in 0.1 mL of media for ^{19}F NMR measurements to assess the cell loading.

PFPE uptake measurement by ^{19}F NMR:

When dendritic cells were used, the cell suspension (0.1 mL) was mixed with 100 ul of 1% TFA solution in glycerol as a calibrated 19F reference. The two solutions were mixed well and transferred into a 5 mm NMR tube. Cell uptake was calculated from the relative areas under the peak at -91.5 ppm (PFPE main peak) and the TFA internal standard peak at -76 ppm. Cellular uptake (i.e., the parameter Fc) was calculated as the mean number of fluorine atoms per single viable cell.

Uptake of PFPE/Pluronic™ emulsions:

Examples of how different PFPE derivatives affect cell uptake when formulated the same way with L35 is shown in Figure 32B. For example, modification of PFPE ester to nonhydrolizable amides (morpholino or diethylamide) clearly improved both cellular uptake and was less toxic then the PFPE-ester/L35 emulsion.

Uptake of PFPE facilitated by protamine sulfate in phagocytic cells:

To further improve uptake in dendritic cells we used protamine sulfate. Protamine sulfate solution was mixed with prepared emulsion diluted in serum free media. After a 30

min. incubation at room temperature, the cells were treated with this mixture for three hours. When protamine sulfate was added to diluted emulsions, the emulsion particle size increased slightly (Figure 18), but the presence of the positively charged amino groups on the droplet surface facilitated cellular uptake. Both PFPE amide/L35 and BODIPy-PFPE amide/L35 emulsion uptake was improved several fold when protamine sulfate was used, as shown in Figure 33. Cell viability was excellent and no morphological change in labeled versus non-labeled cells was observed by light microscopy.

PFPE-tyramide facilitated uptake:

PFPE-tyramide is a novel PFPE derivative designed to promote cellular uptake. PFPE-tyramide emulsifies easily with F68 using the low energy methods described above. PFPE tyramide was non-toxic over various doses and the ^{19}F NMR-measured uptake showed a linear dose dependence (R^2=0.93), as shown in Figure 34. Uptake was comparable to the cell labeling experiments that were facilitated with protamine sulfate. This is the first PFPE derivative designed to be self-deliverable into target cells by PFPE-conjugation to a small molecule. PFPE-tyramide also serves as a platform for many additional conjugation formulations where PFPE can be targeted to specific cell types in culture or in the body by small molecules and peptides and shows the feasibility of PFPE targeting.

4. PFPE labeling of cells in suspension

PFPE amide 1 and PFPE oxide 1a emulsions were used to label cells in suspension. These emulsions showed a dose dependent labeling as measured by ^{19}F NMR. For example, most immune cells when cultured *in vitro* grow in suspension. For successful cell labeling in suspension particle polydispersity is critical. Cells are mixed with emulsion droplets, and when the size and PDI are small, the mixing is homogenous which facilitates uniform cellular labeling. PFPE amide 1 and PFPE oxide 1a emulsions prepared by microfluidization satisfied these criteria.

Jurkat Cells:

Jurkat cells (ATCC, Manassas, VA) were used as a model cell line to evaluate fluorocarbon labeling of cells in suspension. These cells were maintained according to the

ATCC protocol. The suspended cells were labeled at 1-2x10^6 cells/mL in 1 mL of 20% FBS media by mixing with an emulsion dilution prepared in serum free media. After a 3 h incubation at 37 °C, the cells were spun down at 1200 rpm and washed twice with media. The cells are resuspended in 0.5 mL of media. A portion of the cell suspension (1/9) was used for cell number estimates by Cell Titer Glo. The cell suspension was then spin down, and resuspended in 0.2 mL of trypsin solution in PBS, incubated at room temperature for 2 h and used for ^{19}F NMR measurements. The lysed cell suspension (0.2 mL) was mixed with the reference TFA solution (0.2% v/v) in PBS in a 5 mm NMR tube.

Uptake of PFPE amide 1, PFPE oxide 1a and blended PFPE nanoemulsions facilitated by polyethylenimine (PEI) in non-phagocytic cells:

To further improve uptake in non-phagocytic cells, PEI was used in PFPE microfluidized emulsion preparations. Small amounts of PEI added to the emulsion was not enough to reverse the zeta potential from negative to positive; the zeta potential was -26 mV in presence of PEI. The value of zeta potential is not predictive of the cell uptake capacity of the emulsion; however, the presence of primary amines on the PFPE emulsion droplet surface does significantly enhance cell uptake. A significant effect of PEI on PFPE uptake was observed in Jurkat cells, as shown in Figure 41. PFPE-PEI emulsions showed a linear correlation between 19F/cell and labeling dose of emulsion in medium with an excellent viability profile (Figure 42).

Primary T-cells:

Primary T cells were isolated from the mouse spleen as described in WO2005072780. Cells were activated by IL-2 for 3 days prior to fluorocarbon labeling. T cells were labeled in suspension, washed three times with PBS, and resuspended for 19F NMR measurements. Viability was assessed by Cell Titer Glo, using a correlation curve constructed with non-labeled T cells. The viability of PFPE labeled T cells at the time of labeling was >60%. When labeled cells were plated and followed over 48 h, the viability was comparable to that of the un-labeled control cells.

5.1. Correlating fluorescence to ^{19}F NMR signal in BODIPy-PFPE amide/L35 labeled cells

Introducing BODIPy-TR dye had several advantages over hydrophilic Cy dyes. The most important was the ease of coupling to PFPE. BODIPy-TR with primary amine handle is commercially available (e.g., from Molecular Probes, Eugene , OR) as BODIPy-TR cadaverine. Coupling of this amine to PFPE was easy and highly efficient. BODIPy-TR dye was chosen for its chemical stability and fluorescent properties that allow simultaneous labeling with FITC or Rhodamine labeled antibodies, allowing phenotypic confirmation of cellular type and function.

The improved BODIPy-PFPE label was successfully used for T cell and DC labeling. Blended BODIPy-PFPE amide 16, PFPE amide 1 and PFPE oxide 1a, emulsified by microfluidization with F68 and PEI show clear intracellular localization in both DCs and T cells after 3 h incubation, Figures 56 and 57, respectively.

5.2. Correlating fluorescence to ^{19}F NMR signal in BODIPy-PFPE "blended" oil nanoemulsion labeled Jurkat cells

Jurkat cells labeled with BODIPy-PFPE blended nanoemulsion showed a linear correlation between the fluorescence signal and the ^{19}F NMR-measured uptake. This clearly shows that fluorescence measurements of cells labeled with PFPE conjugated to fluorescent dye can be used to estimate uptake without expensive NMR measurements, or serve as a second independent technique to confirm the NMR measurements. Uptake measured by fluorescence and NMR were consistent and comparable (Figure 58).

In order to correlate fluorescence and ^{19}F NMR signal, it was assumed that there was a linear correlation between fluorescence intensity to cell number labeled with BODIPy-PFPE. Fluorescence signal and NMR signal were normalized by the viable cell number. Correlation was linear with R^2=0.9776 (Figures 39 and 58A). Fluorescence microscopy confirmed efficient and uniform dendritic cell labeling with both FITC PFPE blended nanoemulsion (Figure 56A-B) and BODIPy-PFPE blended nanoemulsion (Figure 56C-D). Also, a high-level of uniform labeling was observed when the BODIPy-PFPE amide/L35 emulsion was combined with protamine sulfate (Figure 40). In both cases, the nanoemulsion localizes in the cytoplasm and no emulsion droplets were observed outside the cells or on the cell surface.

6. Uptake of nanoemulsion prepared with Compound 42

Emulsions of of compound 42 (see Table 1) were tested for cellular uptake in dendritic cells (Figure 63) The increase in amount of surfactant decreased the cell uptake upon a 3 hour incubation, as measured by ^{19}F NMR. The cell viability was unchanged upon exposure to all tested compound 42 nanoemulsions. Nanoemulsion A4 was examined for dose dependent uptake in DCs (Figure 64).

Nanoemulsion A4 was also used to label bone marrow derived immature mouse DCs. These cells were incubated with nanoemulsion A4 for 4 hours, washed and the ^{19}F NMR spectrum of labeled cells was analyzed (Figure 65). Labeled cells showed resonance at -72.5 ppm, which is widely separated from the main peak of PFPE (-91.6ppm).

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

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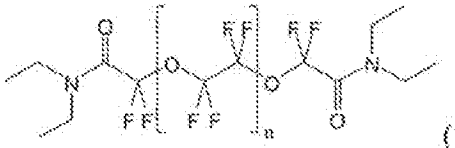
EQUIVALENTS

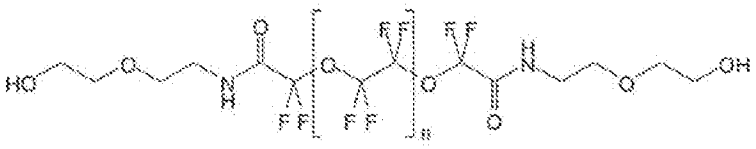
While specific embodiments of the subject inventions are explicitly disclosed herein, the above specification is illustrative and not restrictive. Many variations of the inventions will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the inventions should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

WE CLAIM:

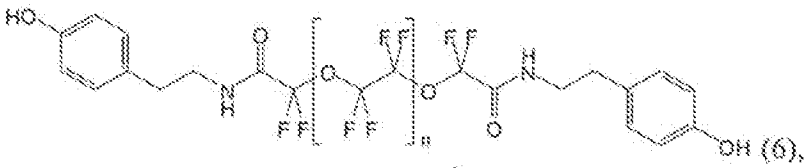
1. A compound of any one of formulae 1, 2, or 6-8:



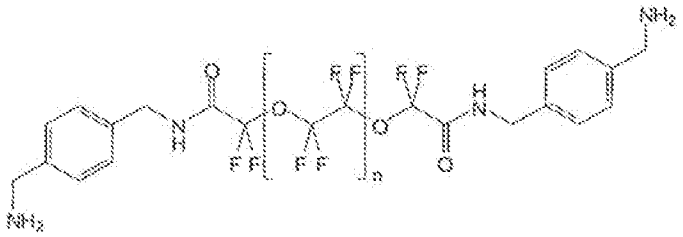
(1),



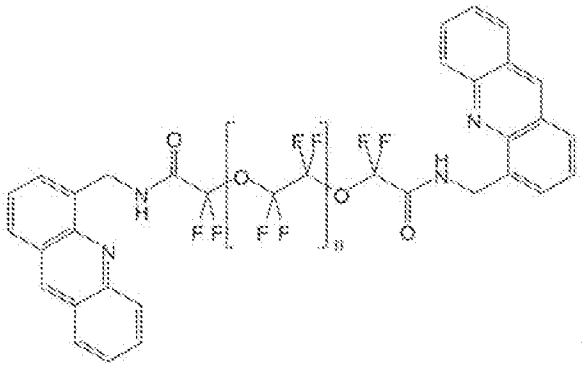
(2),



(6),



(7), or

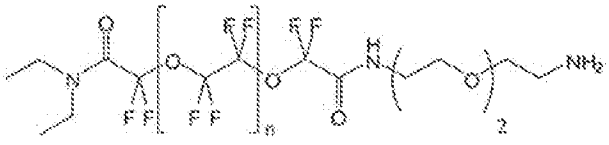


(8),

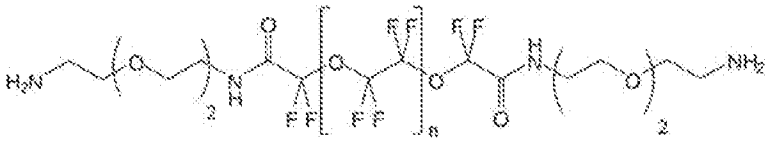
wherein

n, independently for each occurrence, represents an integer from 4 to 16.

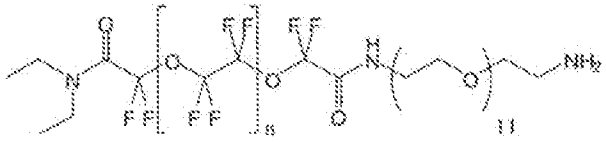
2. A compound of any one of formulae 10-15:



(10),



(11),



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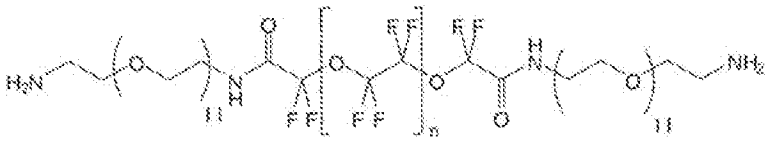

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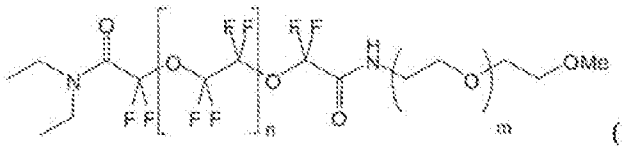
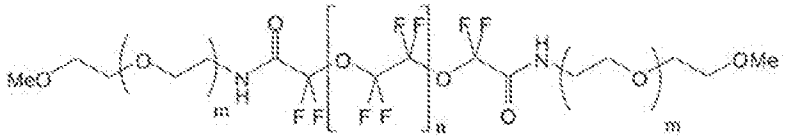


(14), or

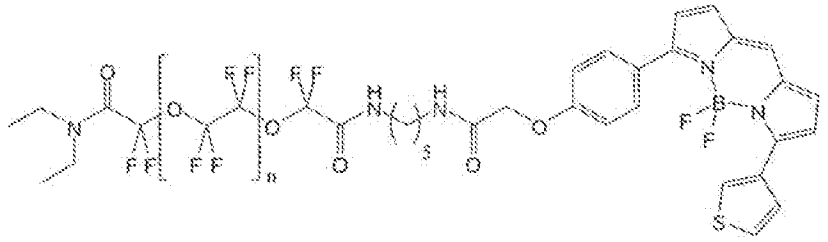


(15),

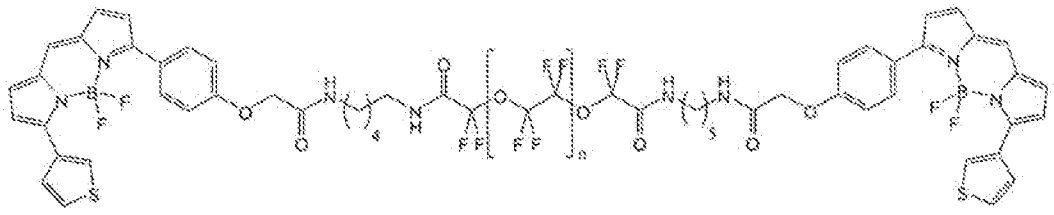
wherein

n, independently for each occurrence, represents an integer from 4 to 16.

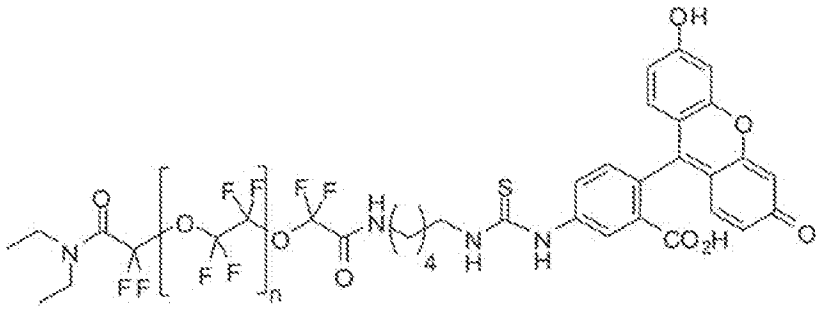
3. A compound of any one of formulae 16-17 or 40-41:



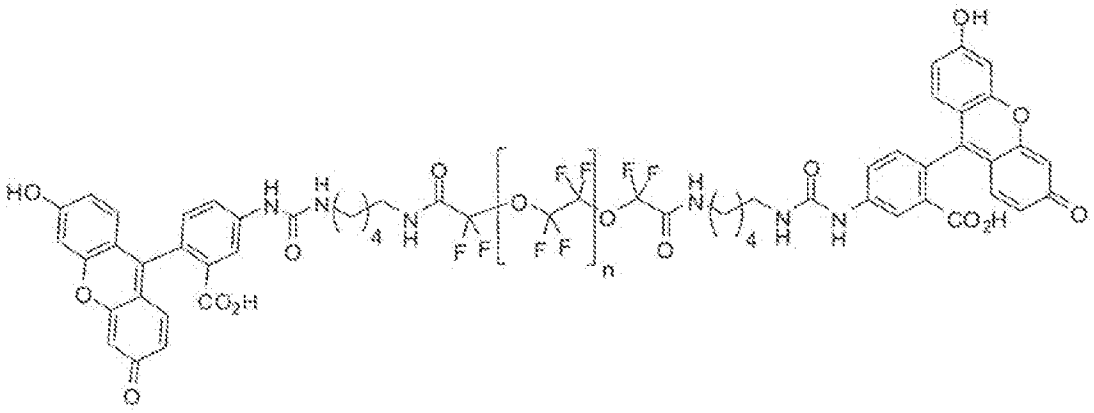
(16),



(17),



(40), or

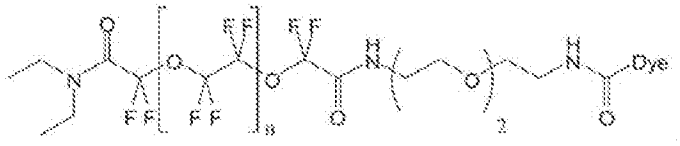


(41)

wherein

n, independently for each occurrence, represents an integer from 4 to 16.

4. A compound of formula 26:



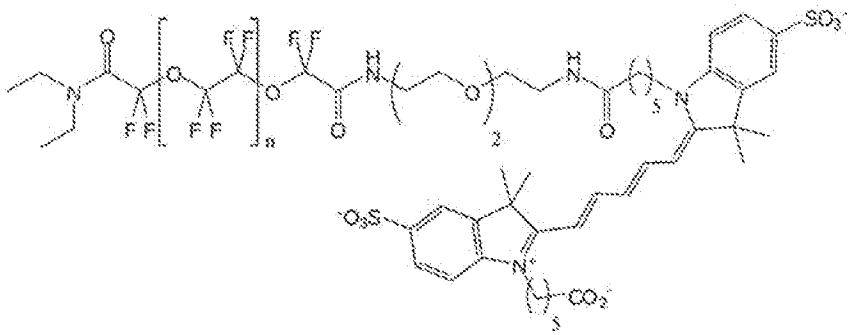
(26),

wherein

n, independently for each occurrence, represents an integer from 4 to 16; and

Dye represents a fluorescent detection moiety.

5. The compound of claim 4, wherein the compound is a compound of formula 20:

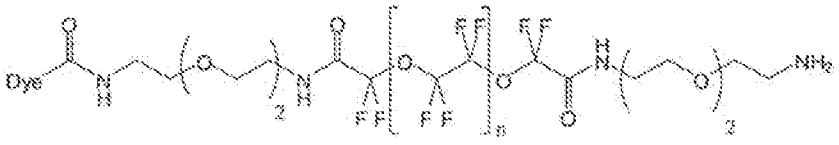


(20),

wherein

n, independently for each occurrence, represents an integer from 4 to 16.

6. A compound of formula 27:



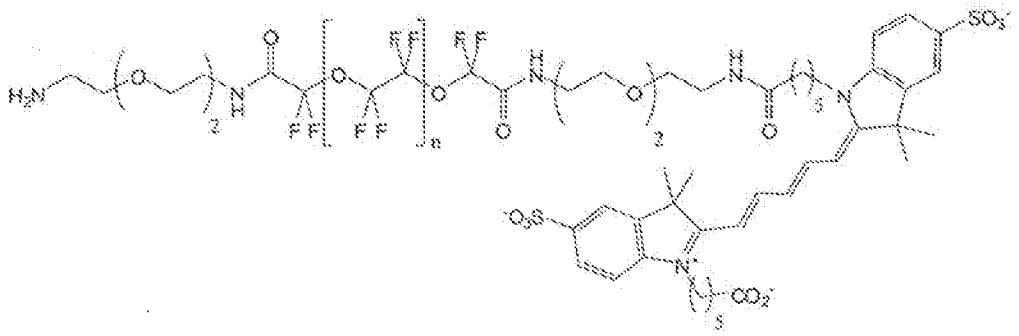
(27),

wherein

n, independently for each occurrence, represents an integer from 4 to 16; and

Dye represents a fluorescent detection moiety.

7. The compound of claim 6, wherein the compound is a compound of formula 21:

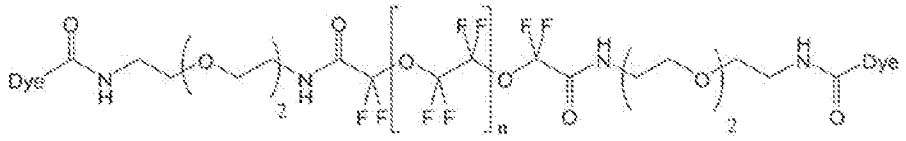


(21),

wherein

n, independently for each occurrence, represents an integer from 4 to 16.

8. A compound of formula 28:



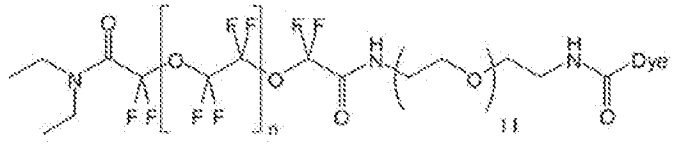
(28),

wherein

n, independently for each occurrence, represents an integer from 4 to 16; and

Dye represents a fluorescent detection moiety.

9. A compound of formula 29:



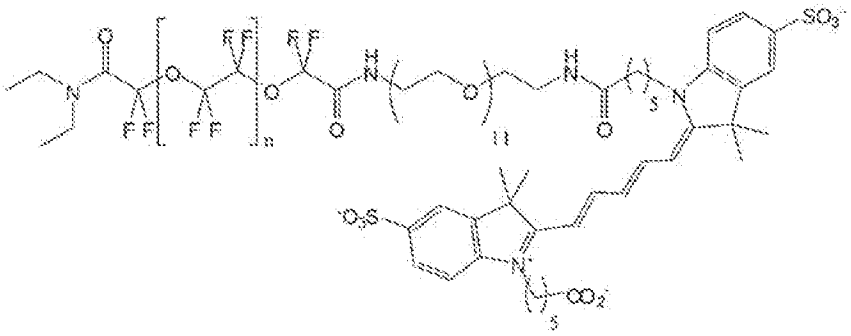
(29),

wherein

n, independently for each occurrence, represents an integer from 4 to 16; and

Dye represents a fluorescent detection moiety.

10. The compound of claim 9, wherein the compound is a compound of formula 23:

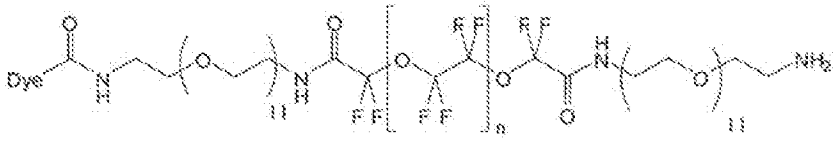


(23),

wherein

n, independently for each occurrence, represents an integer from 4 to 16.

11. A compound of formula 30:



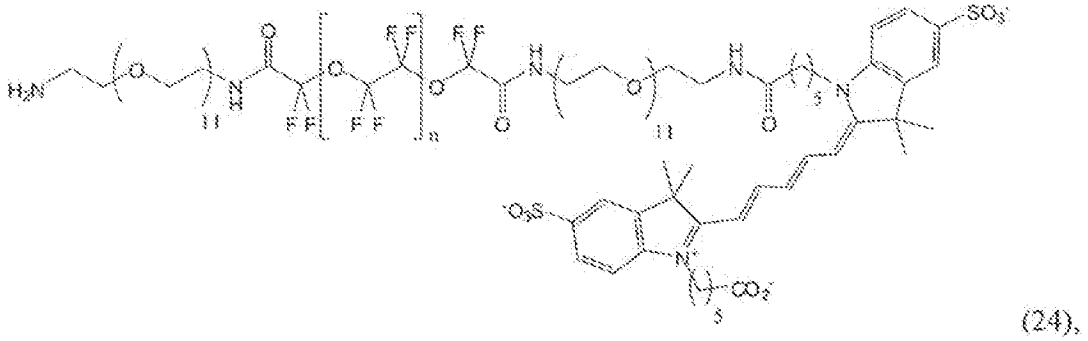
(30),

wherein

n, independently for each occurrence, represents an integer from 4 to 16; and

Dye represents a fluorescent detection moiety.

12. The compound of claim 11, wherein the compound is a compound of formula 24:

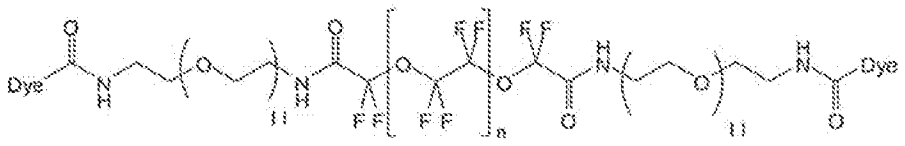


(24),

wherein

n, independently for each occurrence, represents an integer from 4 to 16.

13. A compound of formula 31:



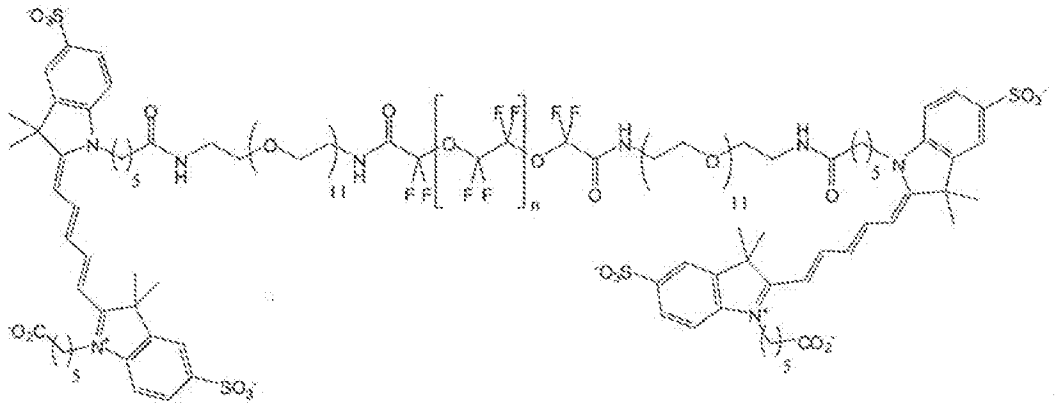
(31),

wherein

n, independently for each occurrence, represents an integer from 4 to 16; and

Dye represents a fluorescent detection moiety.

14. The compound of claim 13, wherein the compound is a compound of formula 25:

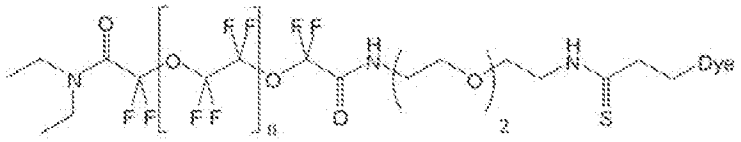


(25),

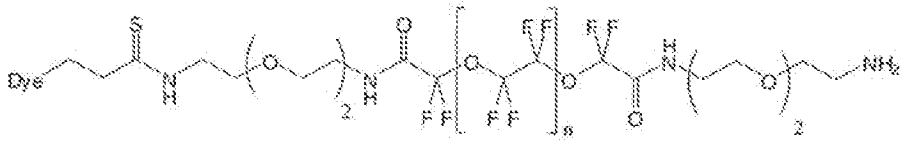
wherein

n, independently for each occurrence, represents an integer from 4 to 16.

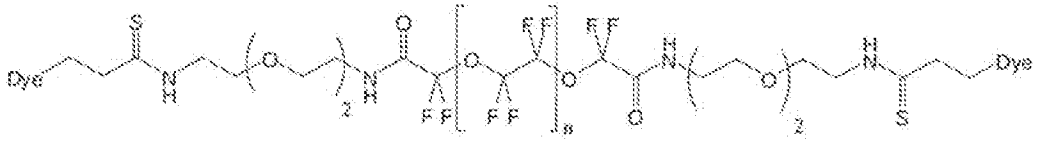
15. A compound of any one of formulae 32-37:



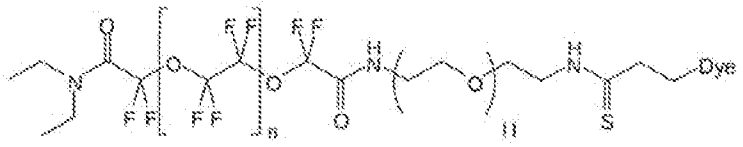
(32),



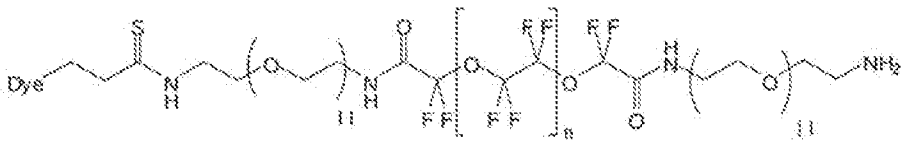
(33),



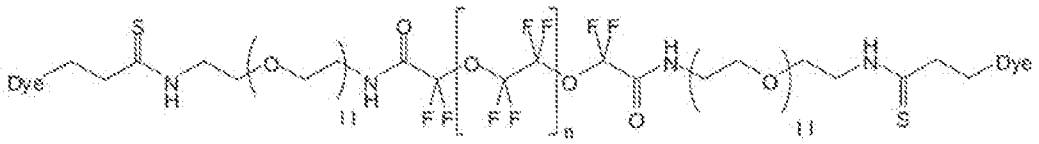
(34),



(35),



(36), or



(37),

wherein

n, independently for each occurrence, represents an integer from 4 to 16; and

Dye, independently for each occurrence, represents a fluorescent detection moiety.

16. A composition comprising a compound of formula 10 and a compound of formula 1.

17. A composition comprising a compound of formula 12 and a compound of formula 1.

18. A composition comprising a compound of formula 14 and a compound of formula 1.

19. A composition comprising a compound of formula 16 and a compound of formula 1.

20. A composition comprising a compound of formula 40 and a compound of formula 1.

21. A composition comprising a compound of formula 10 and a compound of formula 20.

22. A composition comprising a compound of formula 10, a compound of formula 20, and a compound of formula 1.

23. A composition comprising a compound of formula 11 and a compound of formula 21.

24. A composition comprising a compound of formula 11 and a compound of formula 22.

25. A composition comprising a compound of formula 12 and a compound of formula 23.

26. A composition comprising a compound of formula 12, a compound of formula 23, and a compound of formula 1.

27. A composition comprising a compound of formula 13 and a compound of formula 24.

28. A composition comprising a compound of formula 13 and a compound of formula 25.

29. A composition comprising a compound of formula 10 and a compound of formula 26.

30. A composition comprising a compound of formula 10, a compound of formula 26, and a compound of formula 1.

31. A composition comprising a compound of formula 11 and a compound of formula 27.

32. A composition comprising a compound of formula 11 and a compound of formula 28.

33. A composition comprising a compound of formula 12 and a compound of formula 29.

34. A composition comprising a compound of formula 12, a compound of formula 29, and a compound of formula 1.

35. A composition comprising a compound of formula 13 and a compound of formula 30.

36. A composition comprising a compound of formula 13 and a compound of formula 31.

37. A composition comprising a compound of formula 10 and a compound of formula 32.

38. A composition comprising a compound of formula 10, a compound of formula 32, and a compound of formula 1.

39. A composition comprising a compound of formula 11 and a compound of formula 33.

40. A composition comprising a compound of formula 11 and a compound of formula 34.

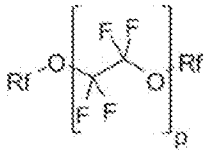
41. A composition comprising a compound of formula 12 and a compound of formula 35.

42. A composition comprising a compound of formula 12, a compound of formula 35, and a compound of formula 1.

43. A composition comprising a compound of formula 13 and a compound of formula 36.

44. A composition comprising a compound of formula 13 and a compound of formula 37.

45. A composition comprising one or more compound of any one of claims 1-15 and a compound of formula 1a,



PFPE oxide, 1a

wherein

p represents an integer from 8 to 13; and

Rf is CF_3 and CF_2CF_3 in a ratio of 2:1.

46. The composition of claim 45, wherein the composition comprises 80-95% v/v of the compound of formula 1a.

47. A composition comprising one or more compound of any one of claims 1-15 and perfluoro-15-crown-5 ether.

48. The composition of claim 47, wherein the composition comprises 80-95% v/v of perfluoro-15-crown-5 ether.

49. A compound of formula:

(42).

50. An emulsion comprising a compound of any one of claims 1-15 or 49 or a composition of any one of claims 16-48.

51. The emulsion of claim 50, further comprising a block copolymer.

52. The emulsion of claim 51, wherein the block copolymer is a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 1900, 2900, or 8400.

53. The emulsion of claim 50, further comprising a lipid.

54. The emulsion of claim 53, wherein the lipid is DMPC.

55. The emulsion of claim 54, further comprising a block copolymer.

56. The emulsion of claim 55, wherein the block copolymer is a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 1900.

57. The emulsion of claim 50, further comprising polyethylamine.

58. The emulsion of claim 50, further comprising protamine sulfate.

59. The emulsion of claim 58, further comprising a block copolymer.

60. The emulsion of claim 59, wherein the block copolymer is a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 1900.

61. The emulsion of claim 50, wherein the emulsion has a mean particle size of less than 200 nM in diameter.

62. The emulsion of claim 50, wherein the emulsion is stable at temperatures ranging from 4 °C to 37 °C.

63. The emulsion of claim 50, wherein the emulsion has a polydispersity index ranging from 0.1 to 0.2.

64. An emulsion selected from the group consisting of :

an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular

weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, a compound of formula 1a, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, a compound of formula 1a, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular

weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, a compound of formula 1a, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, a compound of formula 1a, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1a and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-

poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, perfluoro-15-crown-5 ether, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, perfluoro-15-crown-5 ether, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular

weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, perfluoro-15-crown-5 ether, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1, perfluoro-15-crown-5 ether, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1a, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising a compound of formula 1a, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising a compound of formula 1a, perfluoro-15-crown-5 ether, and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and protamine sulfate;

an emulsion comprising perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29, and polyethylamine;

an emulsion comprising perfluoro-15-crown-5 ether and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an

average molecular weight of 8400, an average number of PEO units of about 153, and an average number of PPO units of about 29;

an emulsion comprising a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate;

an emulsion comprising a compound of formula 1a, a compound of formula 1, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate;

an emulsion comprising a compound of formula 1a, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate;

an emulsion comprising a compound of formula 1a, a compound of formula 1, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate;

an emulsion comprising perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO

units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate;

an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate;

an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, perfluoro-15-crown-5 ether, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate;

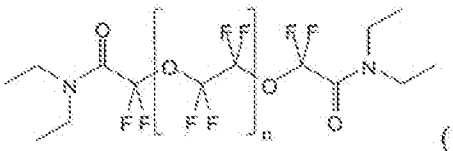
an emulsion comprising a compound of formula 1, a compound of formula 16, a compound of formula 17, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate;

an emulsion comprising a compound of formula 1, a compound of formula 18, a compound of formula 19, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate;

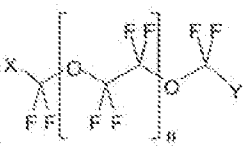
an emulsion comprising a compound of formula 1, a compound of formula 40, a compound of formula 41, a compound of formula 1a, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate; and

an emulsion comprising a compound of formula 42, a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymer with an average molecular weight of 6500, an average number of PEO units of about 74, and an average number of PPO units of about 56, and an emulsifier, wherein the emulsifier is a non-ionic solubiliser comprising glycerol polyethylene glycol ricinoleate.

65. A method for preparing a composition comprising a compound of formula 1 and a compound of formula 38:



(1) and



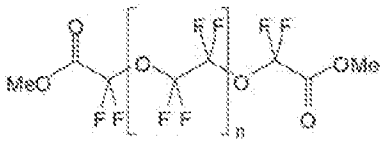
(38),

wherein

n, independently for each occurrence, represents an integer from 4 to 16; and

one or both of X and Y is an amide other than diethyl amide,

comprising:

1) reacting perfluoropolyether methyl ester (39),  (39), having two methyl ester end groups with a primary or secondary aliphatic amine other than diethyl amine;

2) reacting unmodified methyl ester end groups with excess diethyl amine;

3) removing unreacted diethyl amine; and

4) optionally removing non-volatile unreacted amine by selective extraction in fluorinated solvents or fluoropus phase solid extraction and filtration.

66. A method for preparing an emulsion of a PFPE derivative with block copolymer using low energy methods.

67. A method for preparing an emulsion of any one of claims 50-64 comprising low energy methods.

68. A method of any one of claims 66 or 67, wherein the low energy method comprises a thin film method.

69. A method for preparing an emulsion of any one of claims 50-64 comprising high energy methods.

70. The method of claim 69, wherein the high energy method is microfluidization.

71. The method of claim 69, wherein the high energy method is sonication.

72. A method for labeling a cell, the method comprising contacting the cell *ex vivo* with a fluorocarbon imaging reagent comprising a compound of any one of claims 1-15 or 49 under conditions such that the fluorocarbon imaging reagent becomes associated with the cell.

73. A method for detecting a cell in a subject, the method comprising:

a) administering to the subject a cell that is labeled with a fluorocarbon imaging reagent comprising a compound of any one of claims 1-15 or 49; and

b) examining at least a portion of the subject by a nuclear magnetic resonance technique, thereby detecting a labeled cell in the subject.

74. A method for detecting transplanted cells in a transplant recipient, the method comprising:

a) administering cells for transplant to a transplant recipient, at least a portion of which cells for transplant are labeled with a fluorocarbon imaging reagent comprising a compound of any one of claims 1-15 or 49;

b) examining at least a portion of the subject by a nuclear magnetic resonance technique, thereby detecting the labeled cells.

75. A method for quantifying cell number *in vivo*, the method comprising:

a) administering to the subject cells that are labeled with a fluorocarbon imaging reagent comprising a compound of any one of claims 1-15 or 49;

b) examining at least a portion of the subject by a nuclear magnetic resonance technique, thereby detecting labeled cells in the subject; and

c) quantifying the number of labeled cells in a region of interest (ROI).

76. A method for labeling a cell, the method comprising contacting the cell *in vivo* with a fluorocarbon imaging reagent comprising a compound of any one of claims 1-15 or 49 under conditions such that the fluorocarbon imaging reagent becomes associated with the cell.

77. A method for detecting a cell in a subject, the method comprising:

a) administering to the subject a fluorocarbon imaging reagent comprising a compound of any one of claims 1-15 or 49; and

b) examining at least a portion of the subject by a nuclear magnetic resonance technique, thereby detecting a labeled cell in the subject.

78. A labeled cellular formulation for administration to a subject, the formulation comprising:

a) a cell; and

b) a fluorocarbon imaging reagent comprising a compound of any one of claims 1-15 or 49 that is associated with the cell.

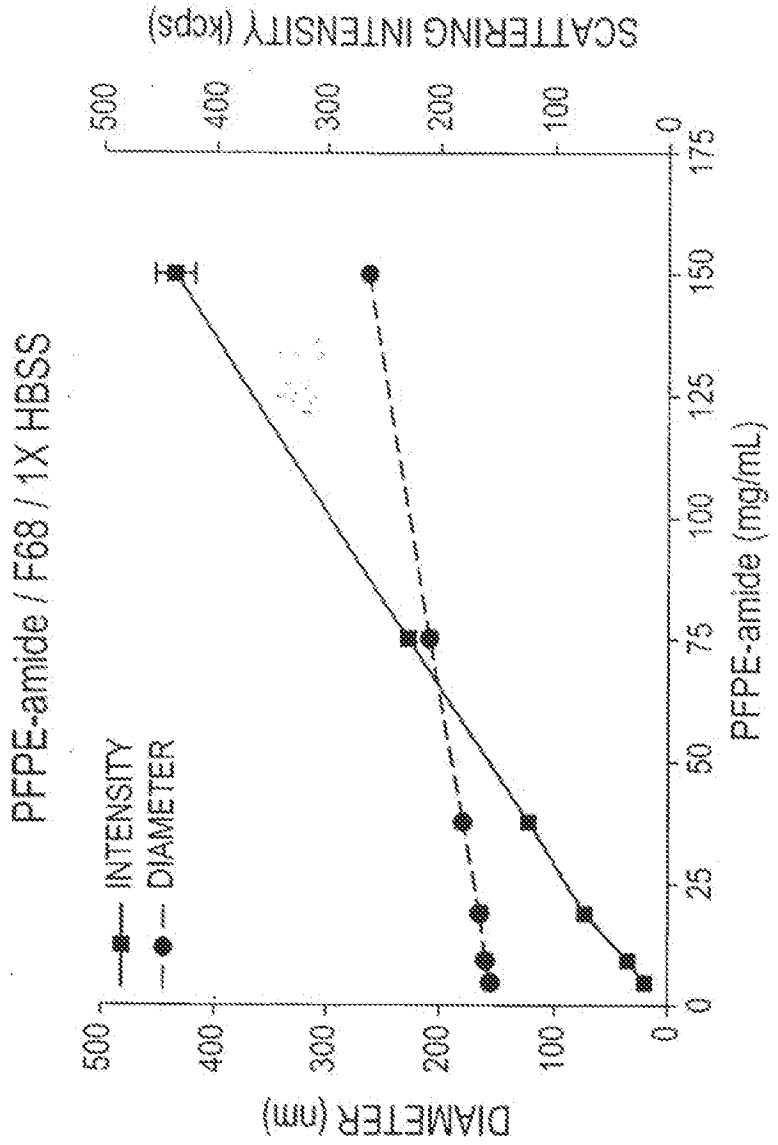

PFPE-amide / F68 / 1X HBSS
INTENSITY
DIAMETER
DIAMETER (nm)
SCATTERING INTENSITY (kcps)
PFPE-amide (mg/mL)
0
25
50
75
100
125
150
175
100
200
300
400
500

Figure 1

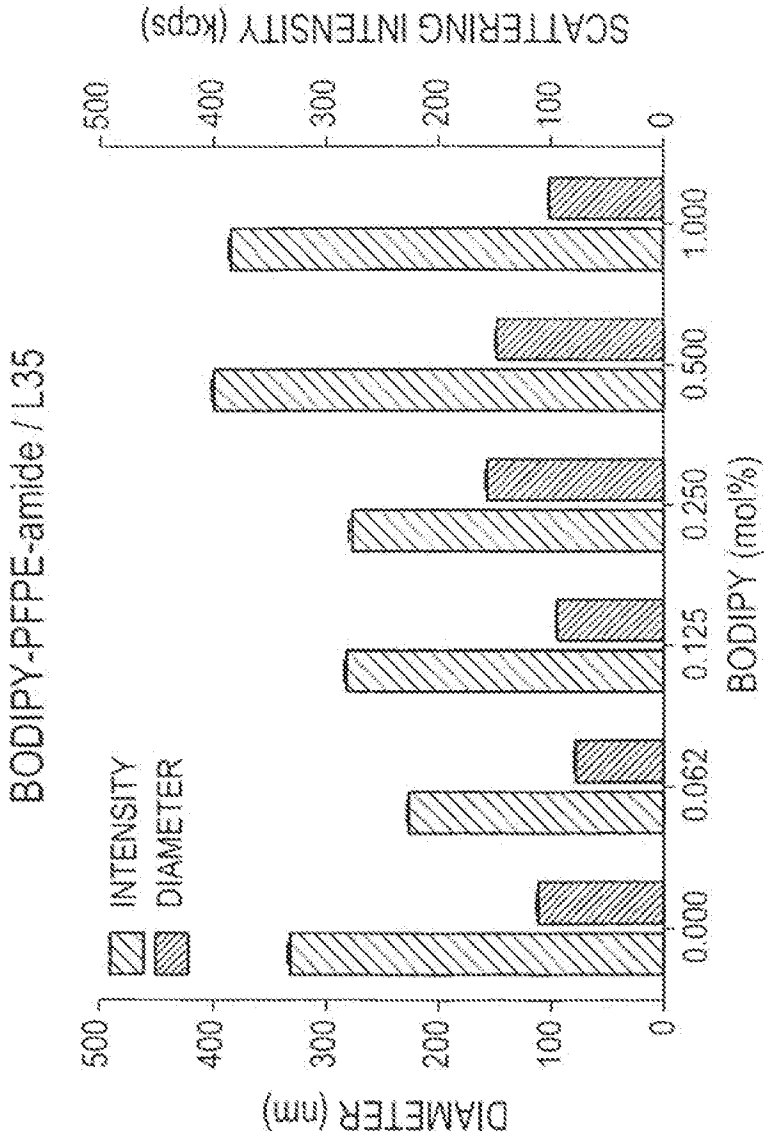

BODIPY-PFPE-amide / L35
INTENSITY
DIAMETER
DIAMETER (nm)
SCATTERING INTENSITY (kcps)
BODIPY (mol%)
0.000
0.062
0.125
0.250
0.500
1.000
0
100
200
300
400
500

Figure 2

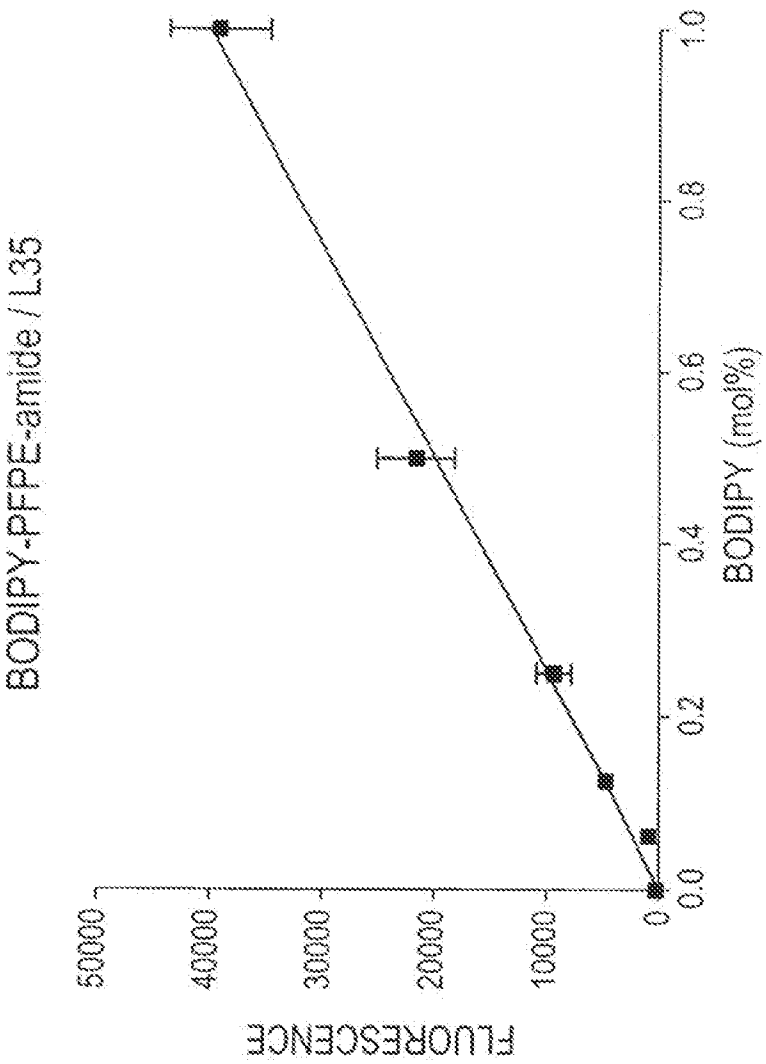

BODIPY-PFPE-amide / L35
FLUORESCENCE
50000
40000
30000
20000
10000
0
0.0
0.2
0.4
0.6
0.8
1.0
BODIPY (mol%)

Figure 3

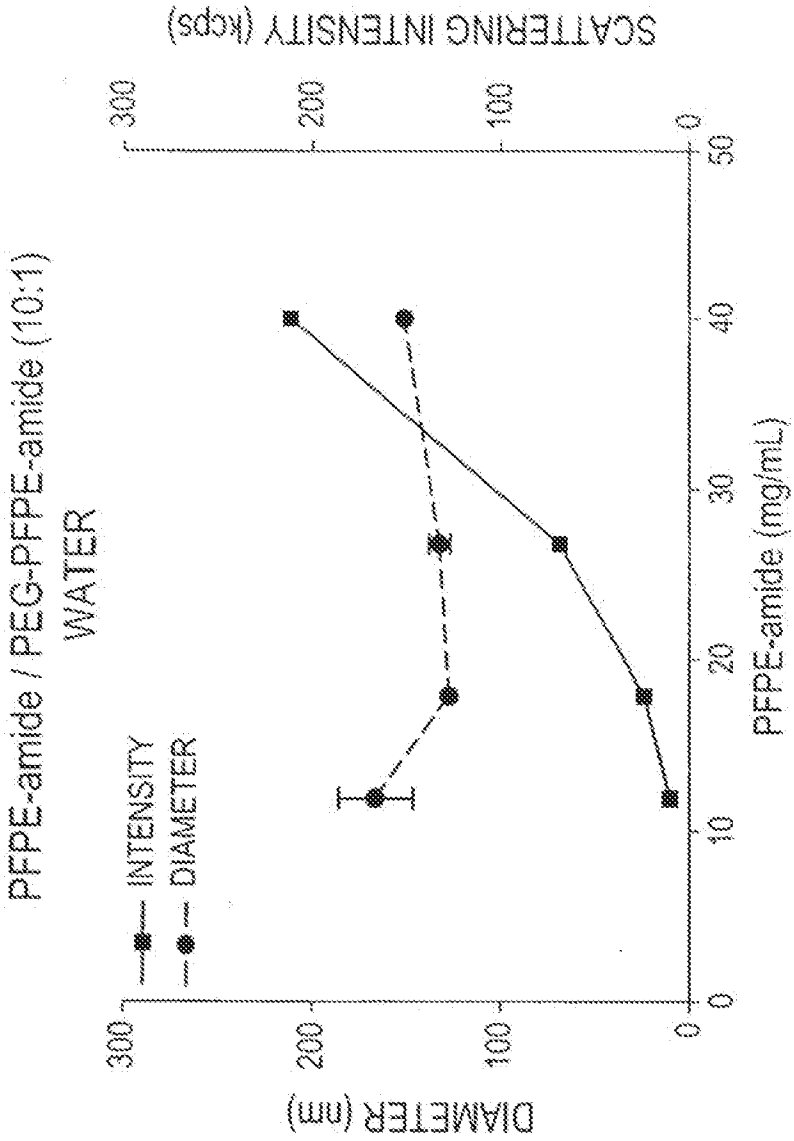

PFPE-amide / PEG-PFPE-amide (10:1)
WATER
INTENSITY
DIAMETER
DIAMETER (nm)
SCATTERING INTENSITY (kcps)
PFPE-amide (mg/mL)
0
100
200
300
0
10
20
30
40
50

Figure 4

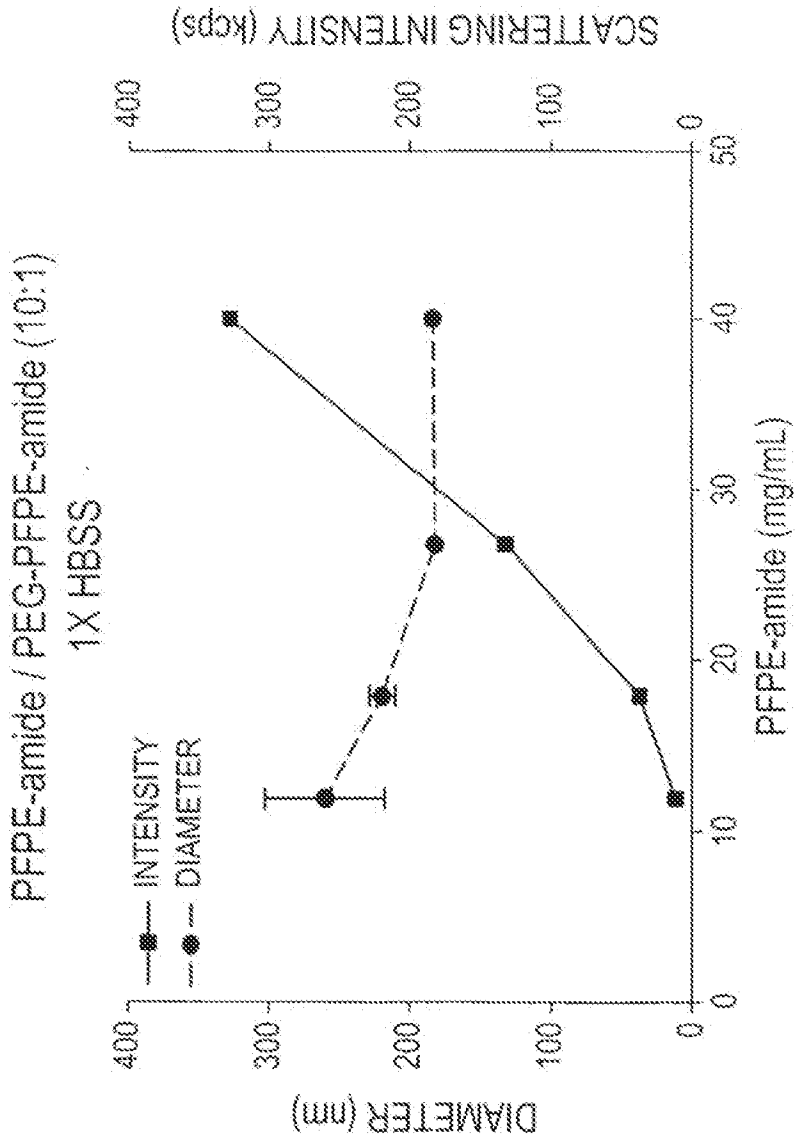

PFPE-amide / PEG-PFPE-amide (10:1)
1X HBSS
INTENSITY
DIAMETER
DIAMETER (nm)
SCATTERING INTENSITY (kcps)
PFPE-amide (mg/mL)
0
100
200
300
400
0
10
20
30
40
50

Figure 5

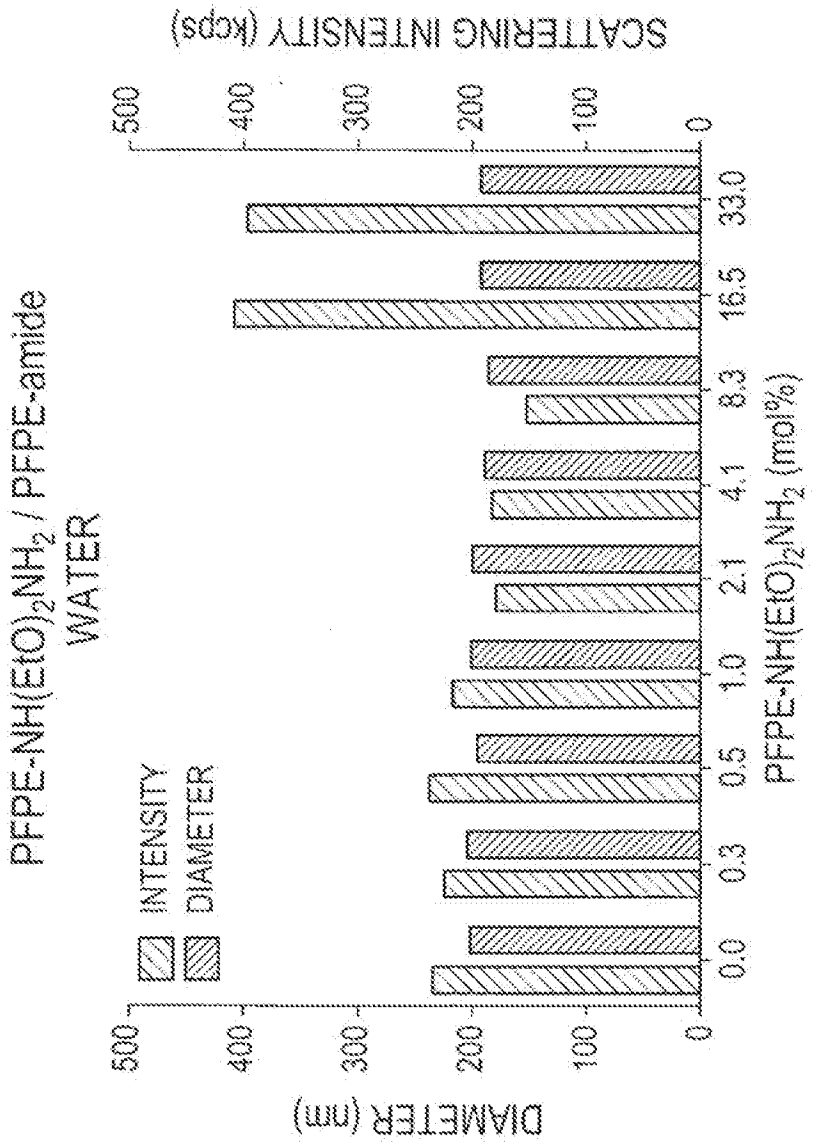

PFPE-NH(EtO)2NH2 / PFPE-amide
WATER
INTENSITY
DIAMETER
DIAMETER (nm)
SCATTERING INTENSITY (kcps)
PFPE-NH(EtO)2NH2 (mol%)
0.0
0.3
0.5
1.0
2.1
4.1
8.3
16.5
33.0
0
100
200
300
400
500

Figure 6

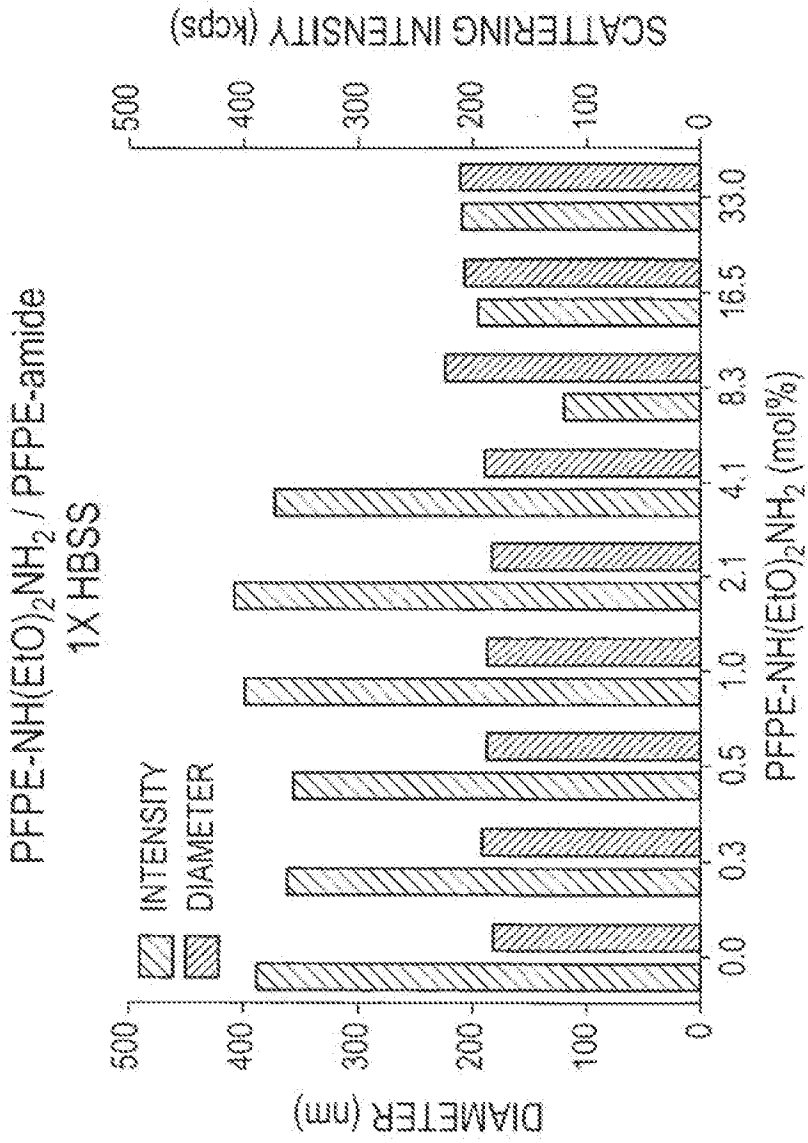

PFPE-NH(EtO)2NH2 / PFPE-amide
1X HBSS
INTENSITY
DIAMETER
DIAMETER (nm)
SCATTERING INTENSITY (kcps)
PFPE-NH(EtO)2NH2 (mol%)
0.0
0.3
0.5
1.0
2.1
4.1
8.3
16.5
33.0
0
100
200
300
400
500

Figure 7

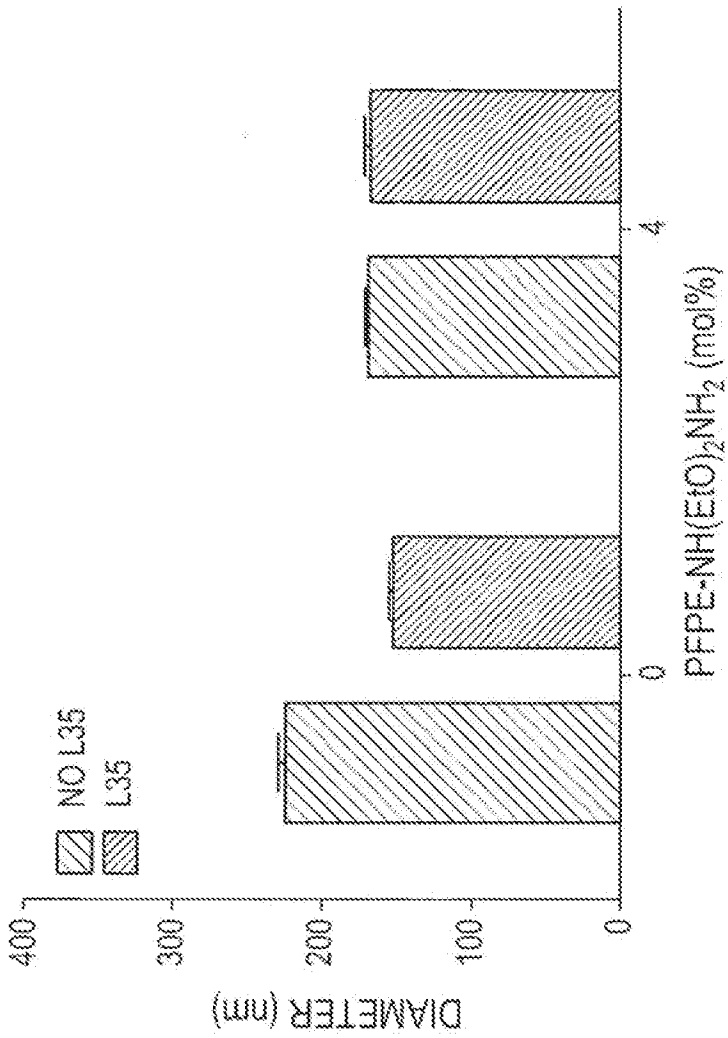

PFPE-NH(EtO)2NH2 / PFPE-amide
1X HBSS
NO L35
L35
DIAMETER (nm)
400
300
200
100
0
0
4
PFPE-NH(EtO)2NH2 (mol%)

Figure 8

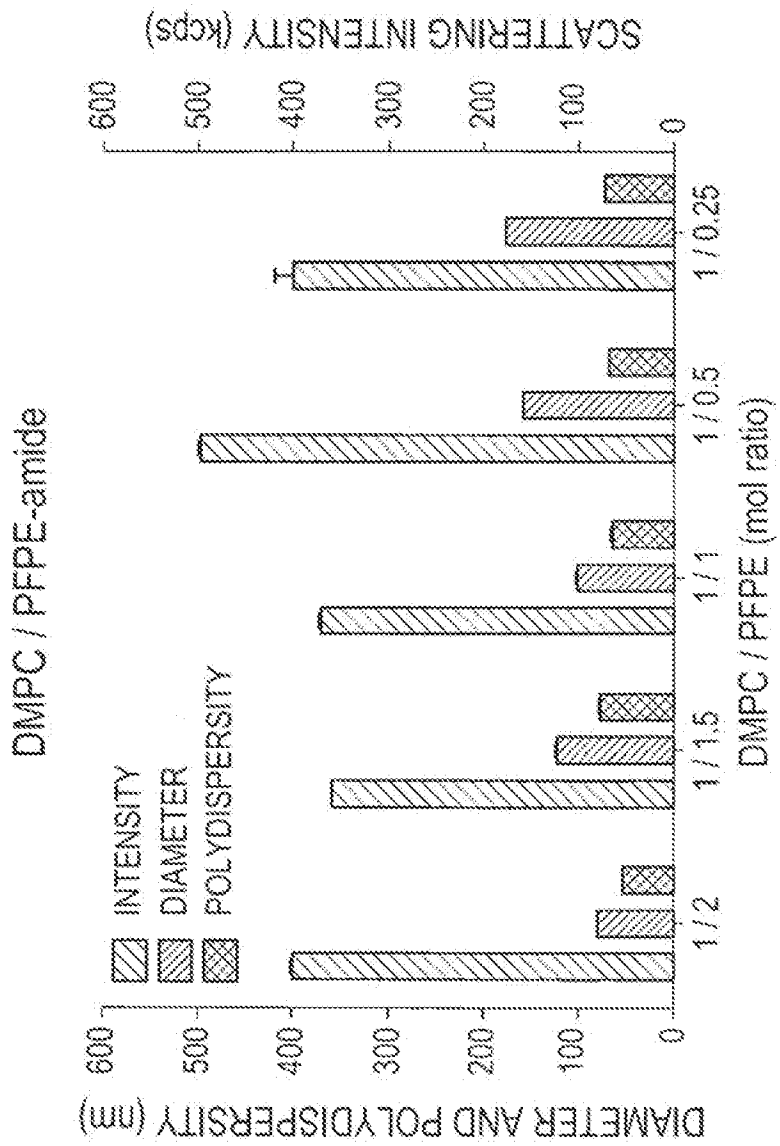

SCATTERING INTENSITY (kcps)
600
500
400
300
200
100
0
1/0.25
1/0.5
1/1
1/1.5
1/2
DMPC / PFPE (mol ratio)
DIAMETER AND POLYDISPERSITY (nm)
INTENSITY
DIAMETER
POLYDISPERSITY
DMPC / PFPE-amide

Figure 9

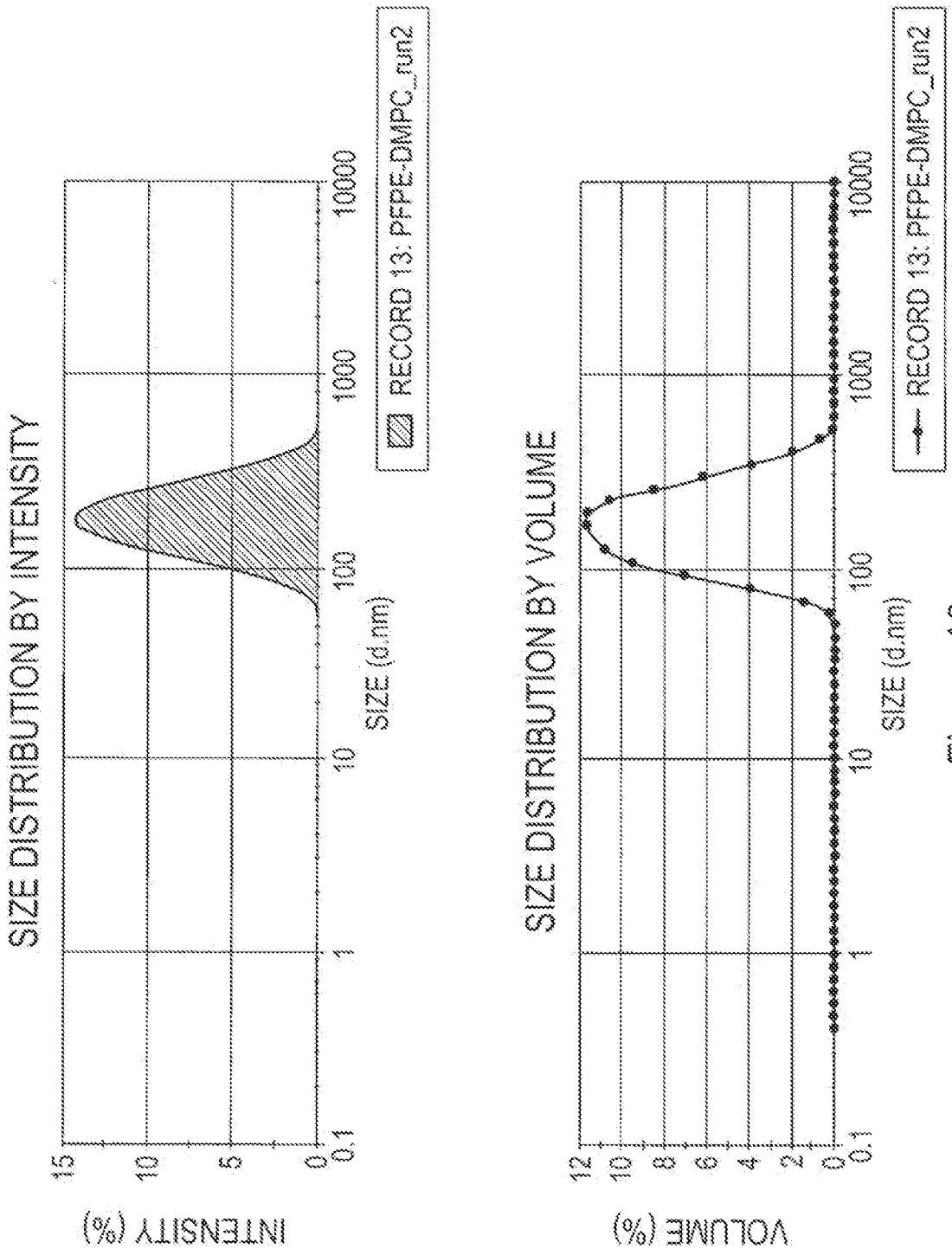

SIZE DISTRIBUTION BY INTENSITY
INTENSITY (%)
15
10
5
0
0.1
1
10
100
1000
10000
SIZE (d.nm)
RECORD 13: PFPE-DMPC_run2
SIZE DISTRIBUTION BY VOLUME
VOLUME (%)
12
10
8
6
4
2
0
0.1
1
10
100
1000
10000
SIZE (d.nm)
RECORD 13: PFPE-DMPC_run2

Figure 10

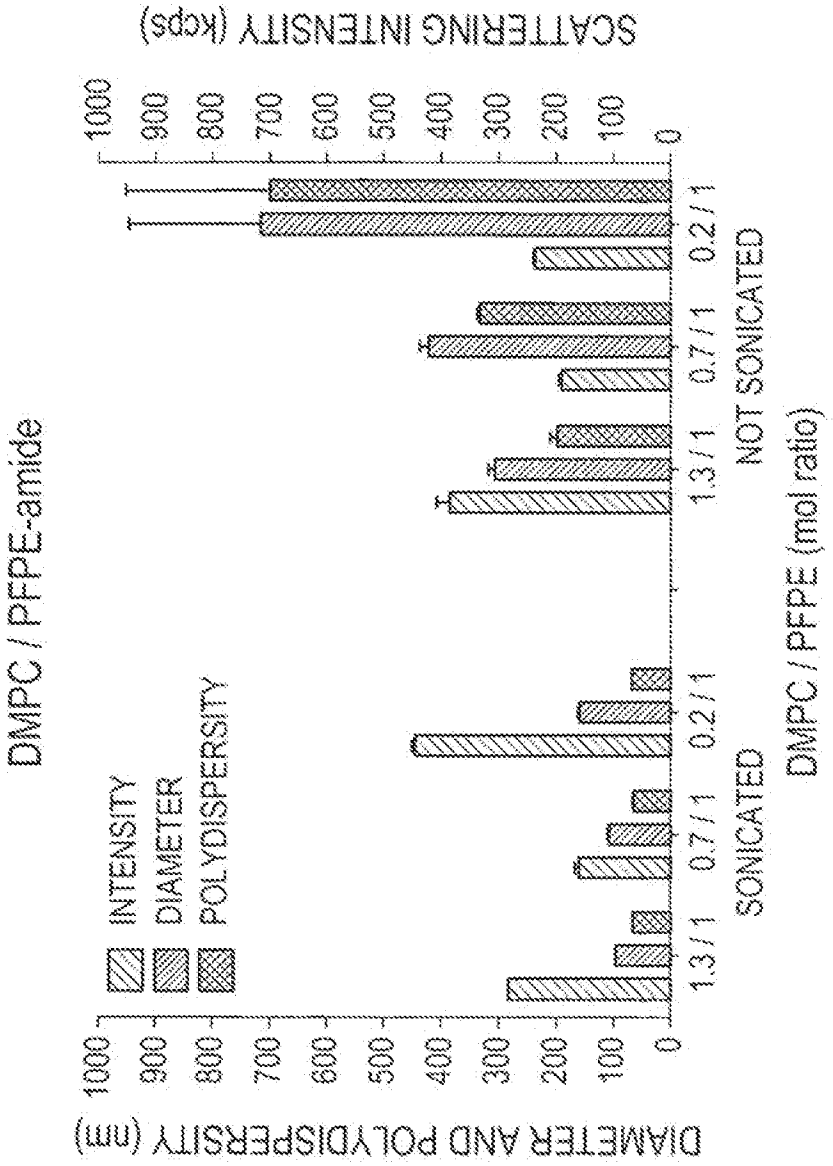

DMPC / PFPE-amide
INTENSITY
DIAMETER
POLYDISPERSITY
DIAMETER AND POLYDISPERSITY (nm)
SCATTERING INTENSITY (kcps)
0
100
200
300
400
500
600
700
800
900
1000
1.3 / 1
0.7 / 1
0.2 / 1
SONICATED
NOT SONICATED
DMPC / PFPE (mol ratio)

Figure 11

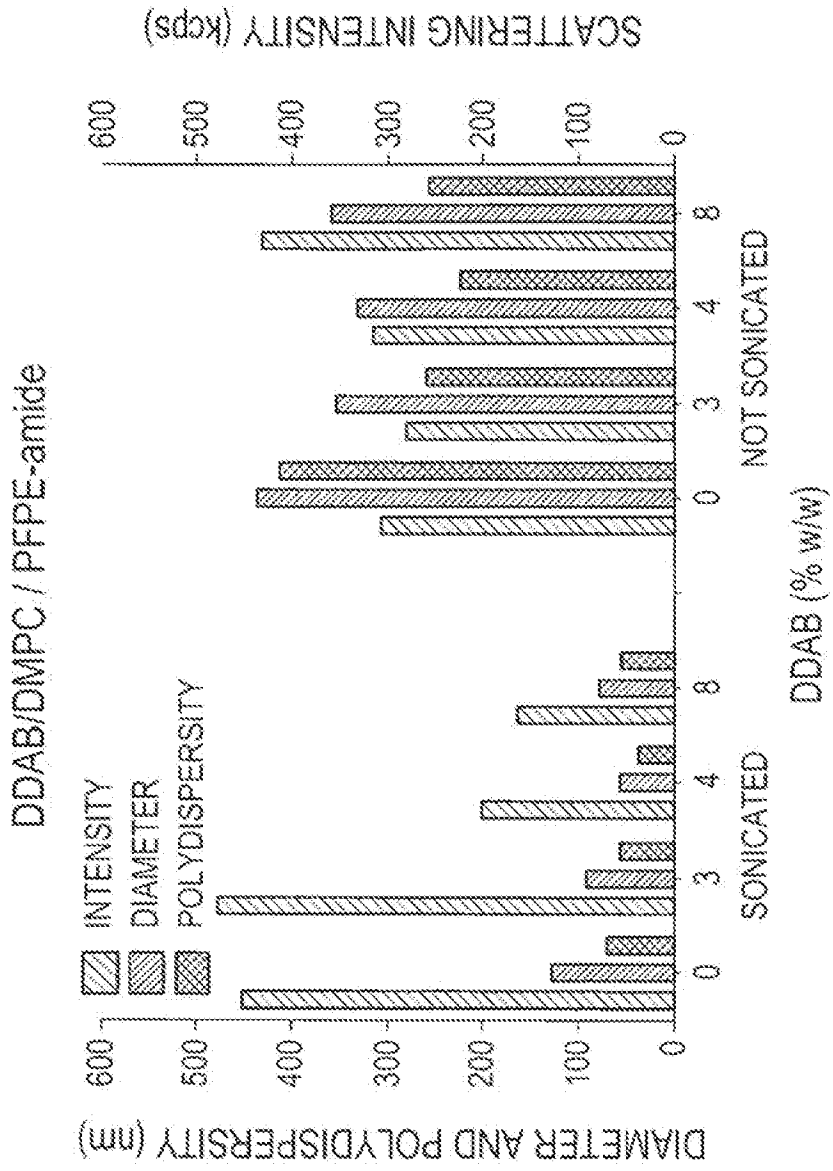

DDAB/DMPC / PFPE-amide
INTENSITY
DIAMETER
POLYDISPERSITY
DIAMETER AND POLYDISPERSITY (nm)
SCATTERING INTENSITY (kcps)
600
500
400
300
200
100
0
SONICATED
NOT SONICATED
0
3
4
8
DDAB (% w/w)

Figure 12

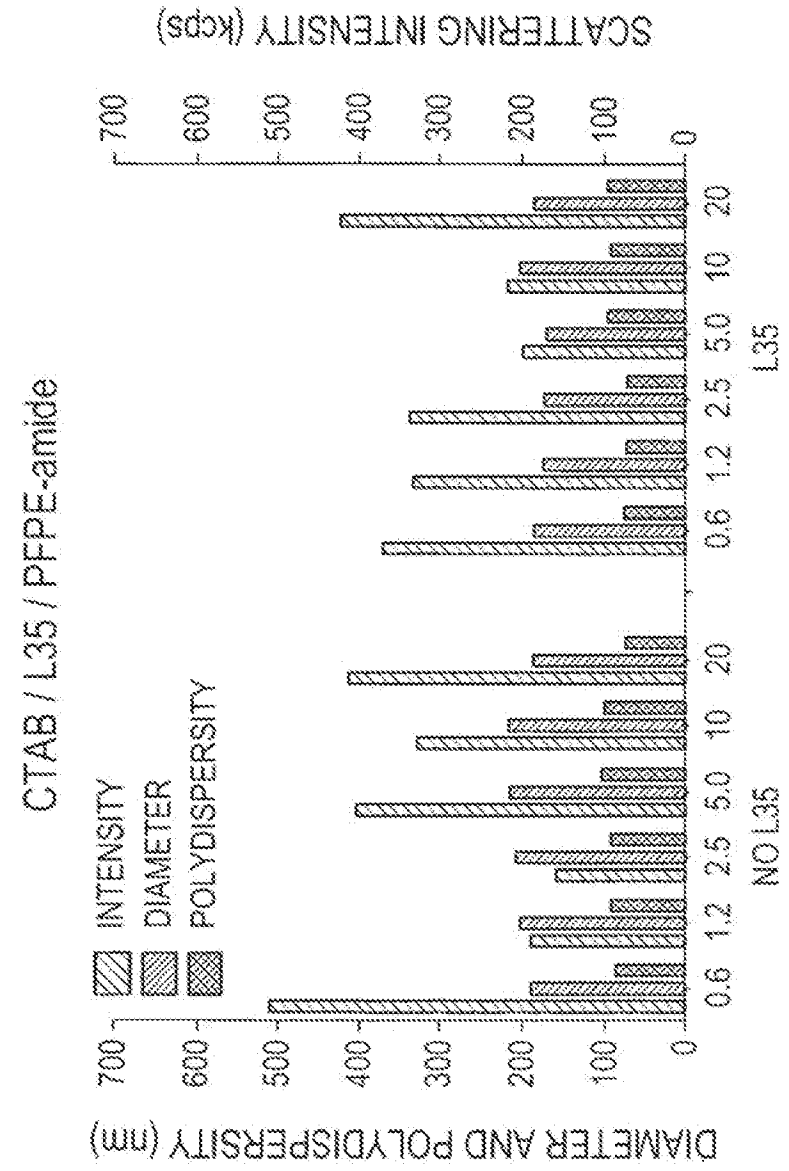

CTAB / L35 / PFPE-amide
INTENSITY
DIAMETER
POLYDISPERSITY
DIAMETER AND POLYDISPERSITY (nm)
SCATTERING INTENSITY (kcps)
700
600
500
400
300
200
100
0
NO L35
0.6
1.2
2.5
5.0
10
20
L35
CTAB (% w/w)

Figure 13

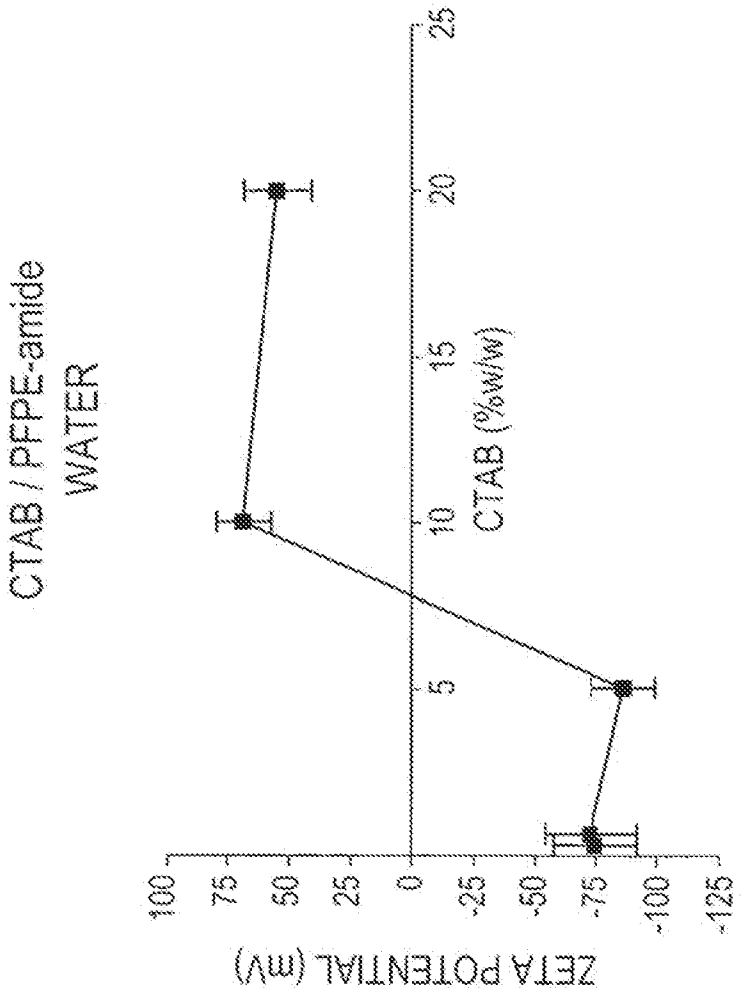

CTAB / PFPE-amide
WATER
ZETA POTENTIAL (mV)
100
75
50
25
0
-25
-50
-75
-100
-125
5
10
15
20
25
CTAB (%w/w)

Figure 14

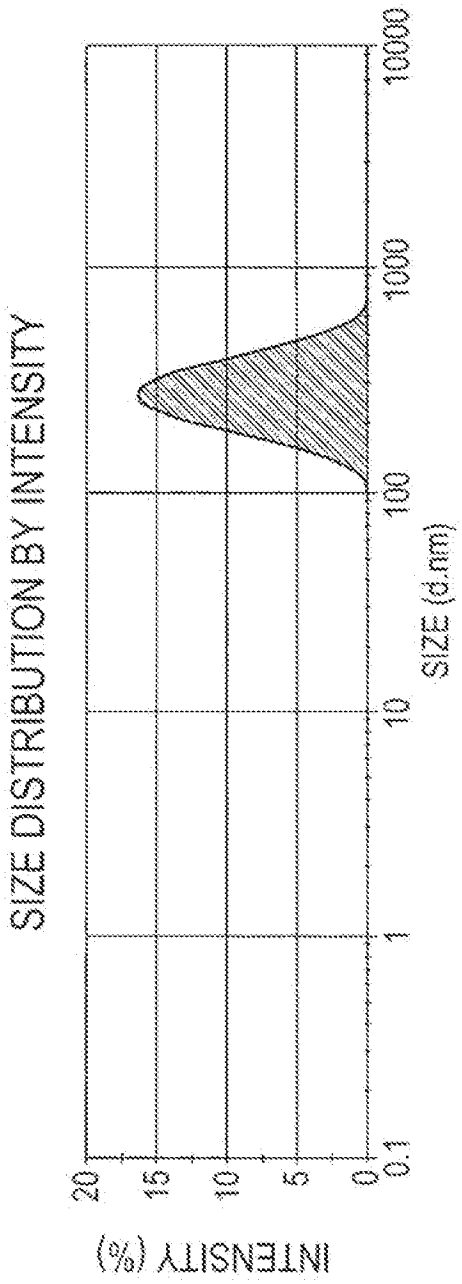

SIZE DISTRIBUTION BY INTENSITY
INTENSITY (%)
20
15
10
5
0
0.1
1
10
100
1000
10000
SIZE (d.nm)

Figure 15

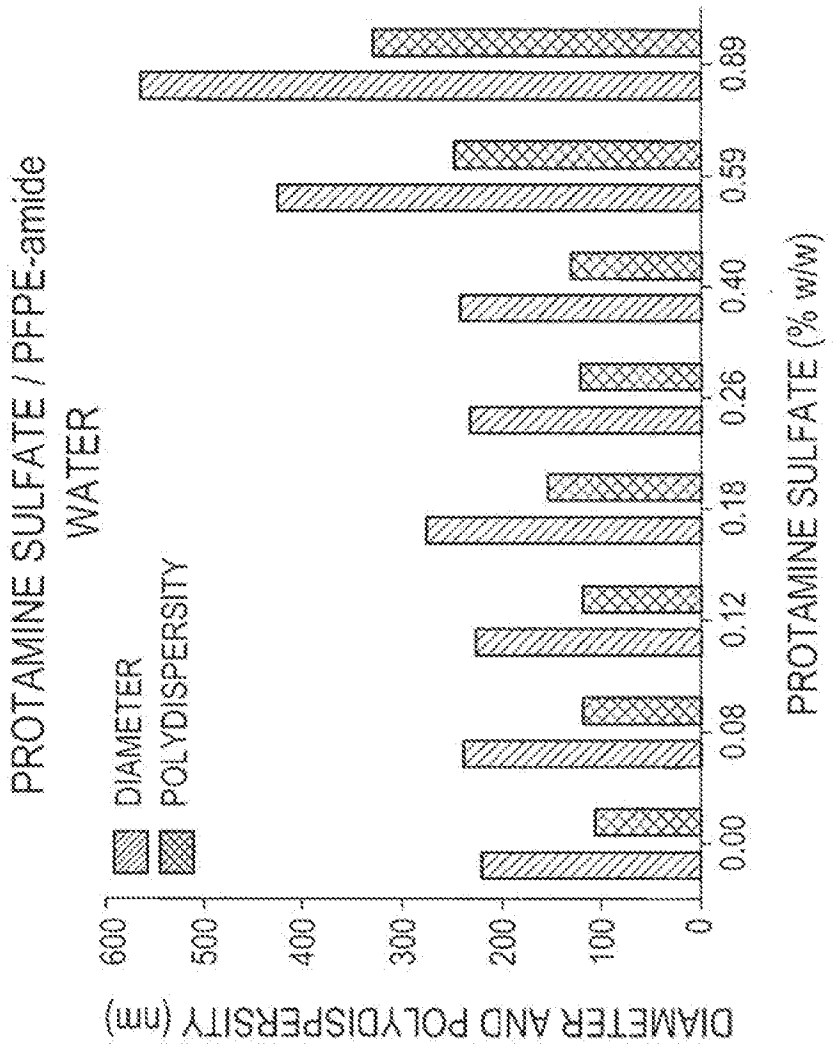

PROTAMINE SULFATE / PFPE-amide
WATER
DIAMETER
POLYDISPERSITY
DIAMETER AND POLYDISPERSITY (nm)
600
500
400
300
200
100
0
0.00
0.08
0.12
0.18
0.26
0.40
0.59
0.89
PROTAMINE SULFATE (% w/w)

Figure 16

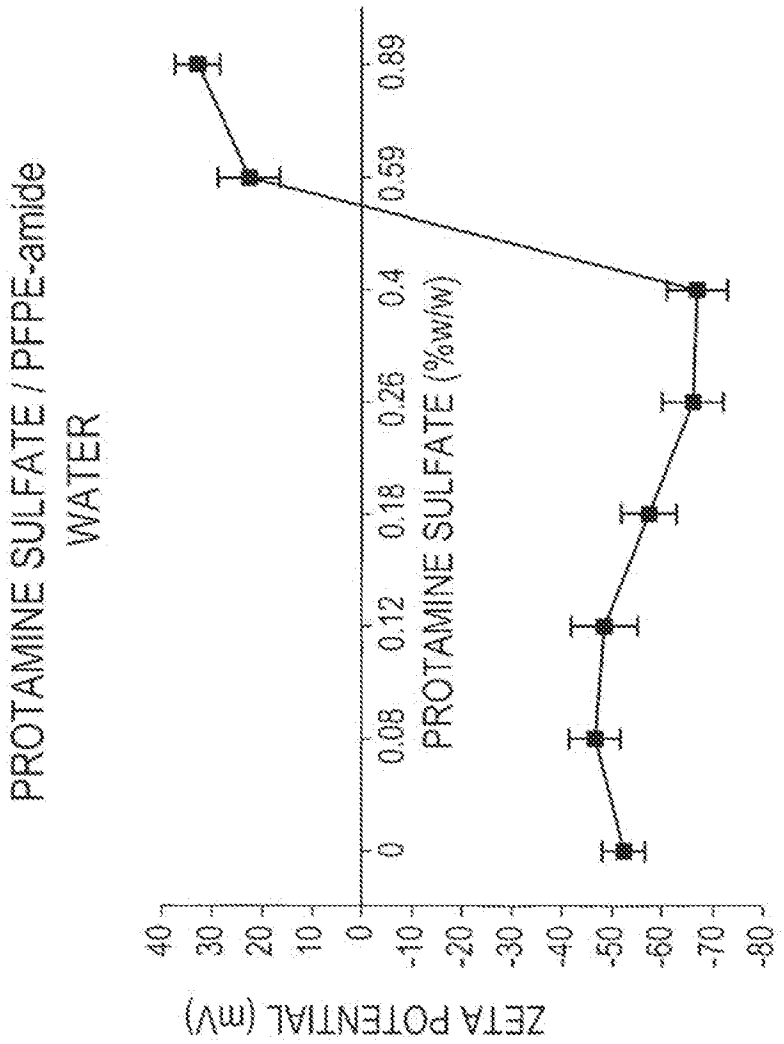

PROTAMINE SULFATE / PFPE-amide
WATER
ZETA POTENTIAL (mV)
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
0
0.08
0.12
0.18
0.26
0.4
0.59
0.89
PROTAMINE SULFATE (%w/w)

Figure 17

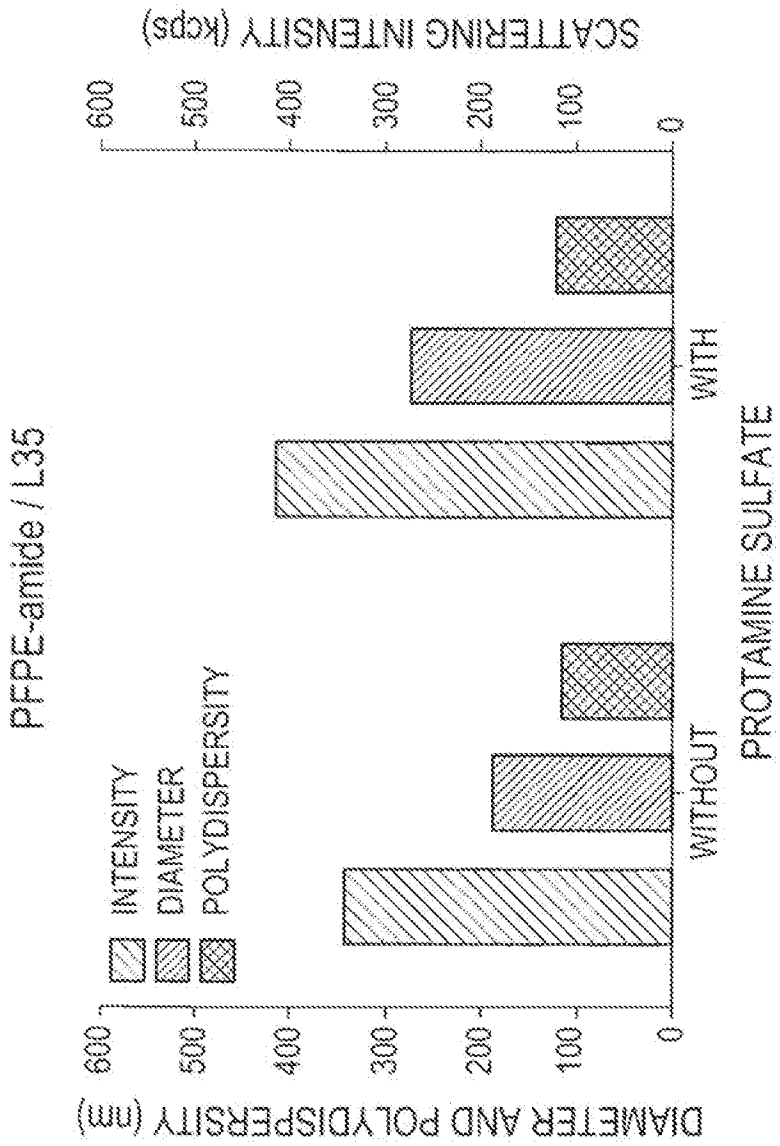

PFPE-amide / L35
INTENSITY
DIAMETER
POLYDISPERSITY
DIAMETER AND POLYDISPERSITY (nm)
SCATTERING INTENSITY (kcps)
600
500
400
300
200
100
0
WITHOUT
WITH
PROTAMINE SULFATE

Figure 18

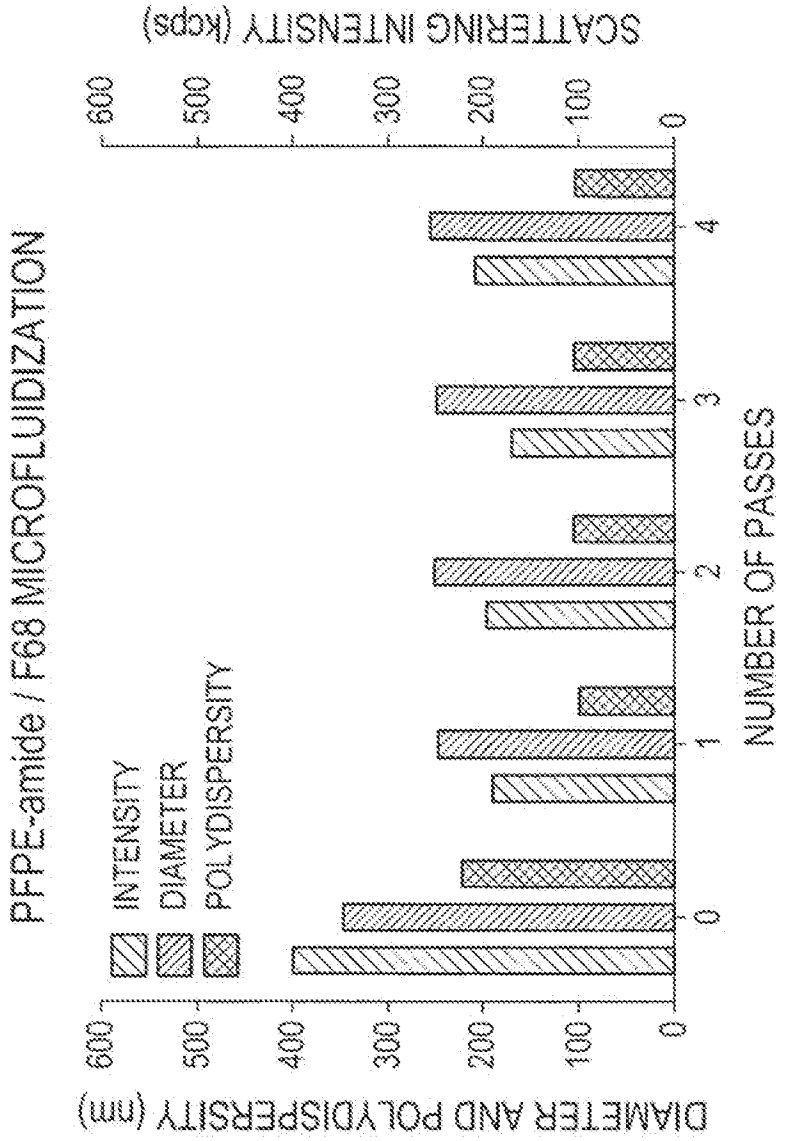

PFPE-amide / F68 MICROFLUIDIZATION
INTENSITY
DIAMETER
POLYDISPERSITY
DIAMETER AND POLYDISPERSITY (nm)
SCATTERING INTENSITY (kcps)
NUMBER OF PASSES
0
100
200
300
400
500
600
0
1
2
3
4

Figure 19

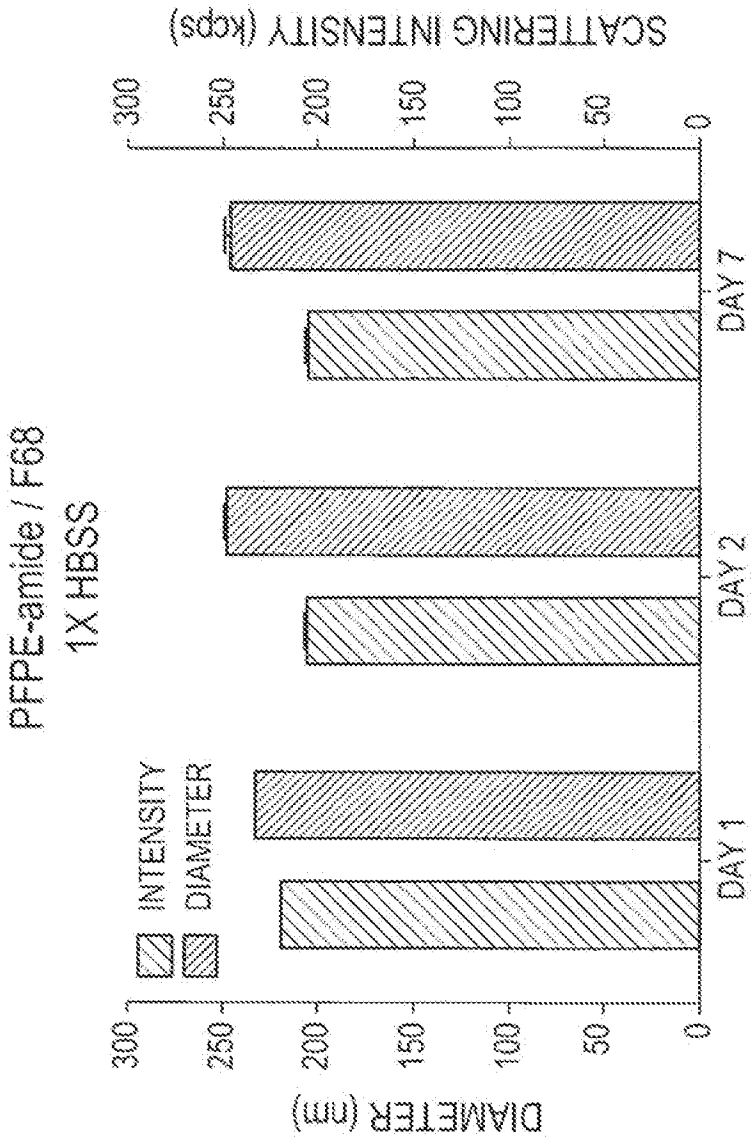

PFPE-amide / F68
1X HBSS
INTENSITY
DIAMETER
DAY 1
DAY 2
DAY 7
DIAMETER (nm)
SCATTERING INTENSITY (kcps)
0
50
100
150
200
250
300

Figure 20

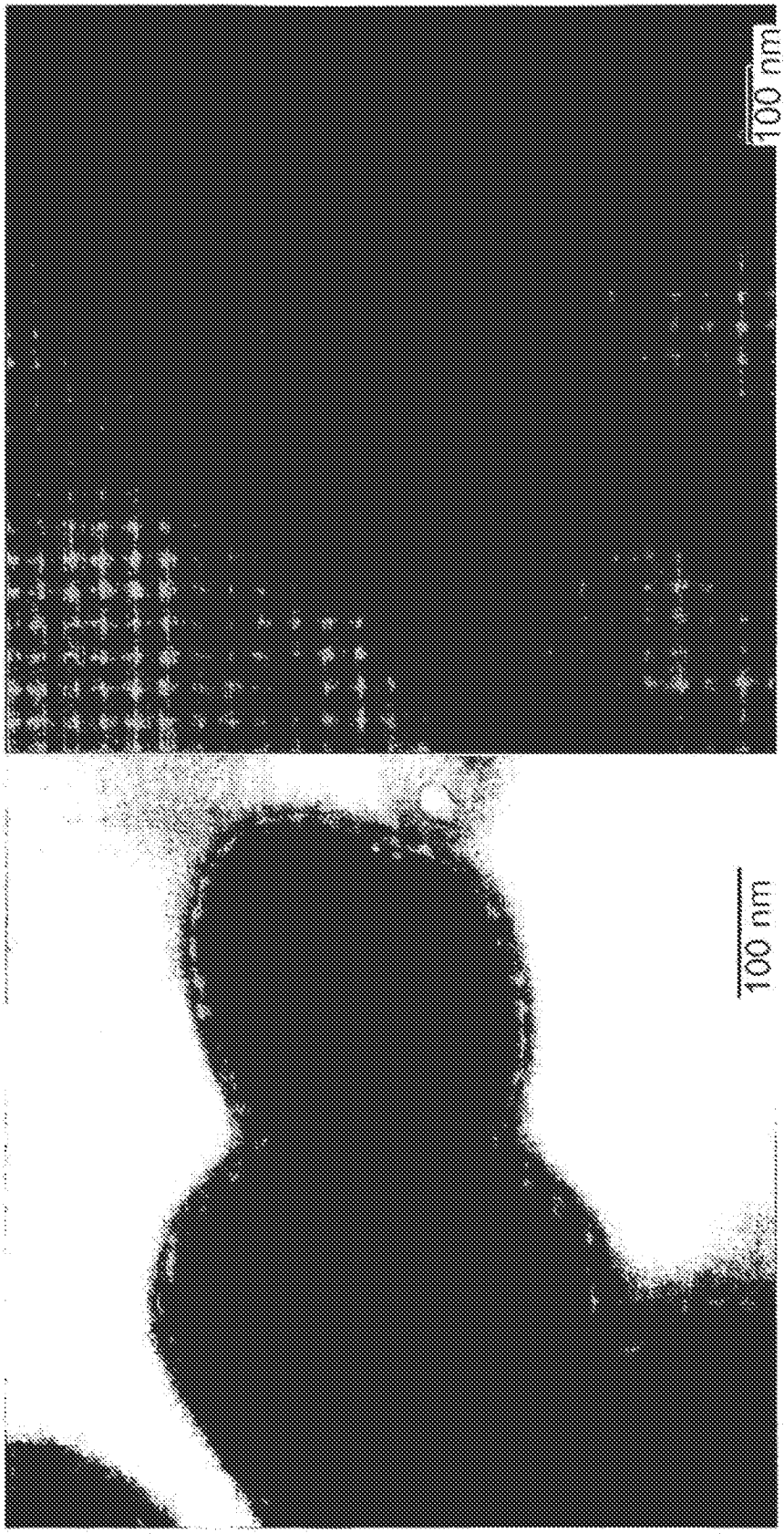


Figure 21

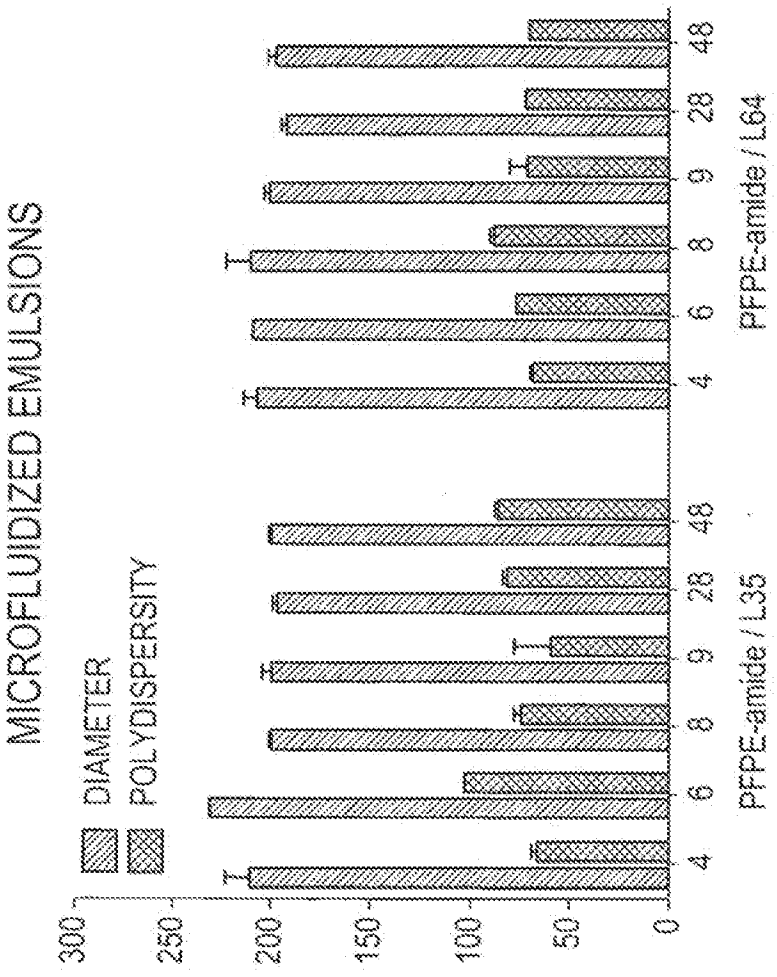

MICROFLUIDIZED EMULSIONS
DIAMETER
POLYDISPERSITY
300
250
200
150
100
50
0
4
6
8
9
28
48
PFPE-amide / L35
DAYS
4
6
8
9
28
48
PFPE-amide / L64

Figure 22

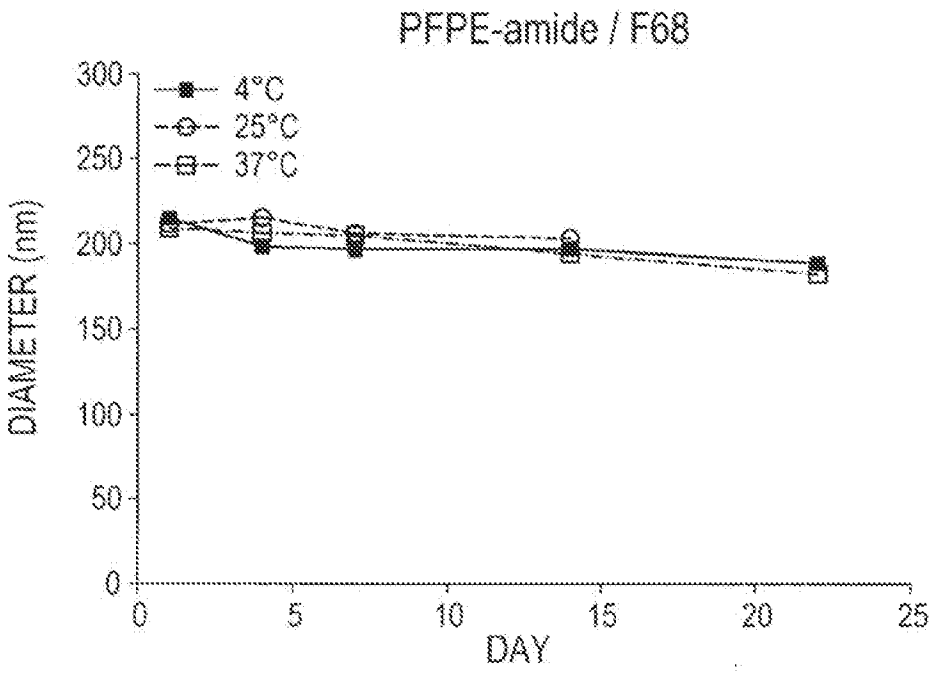


Figure 23A

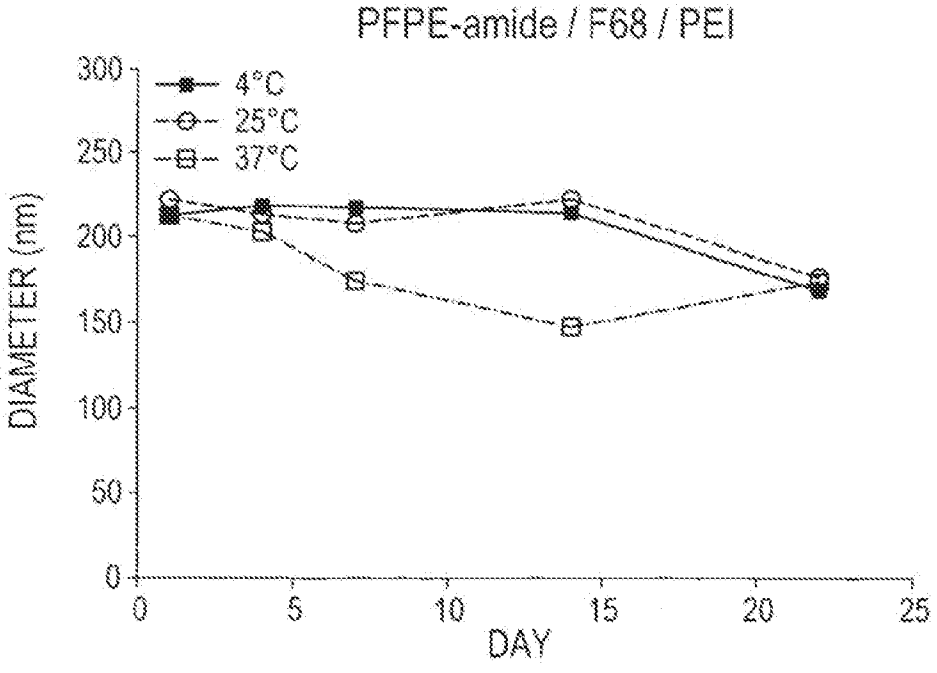


Figure 23B

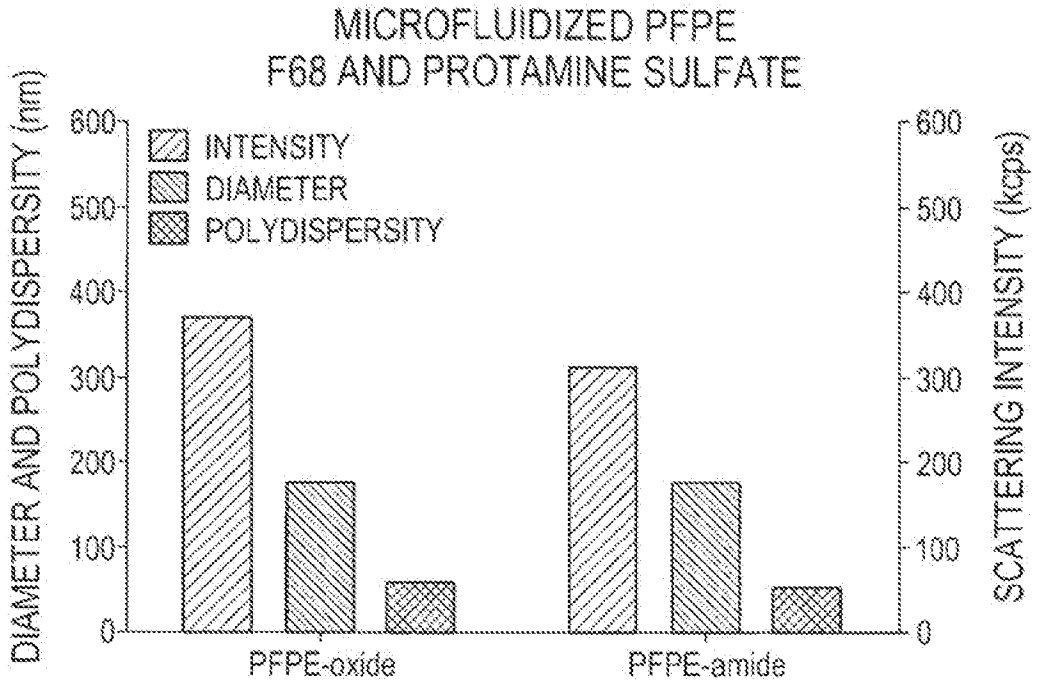


Figure 24A

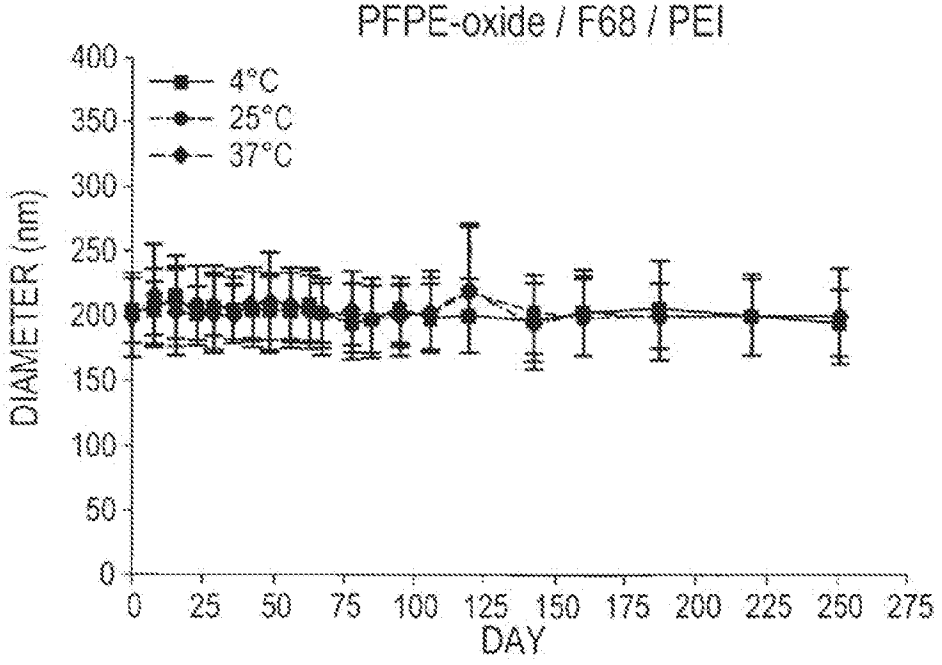


Figure 24B

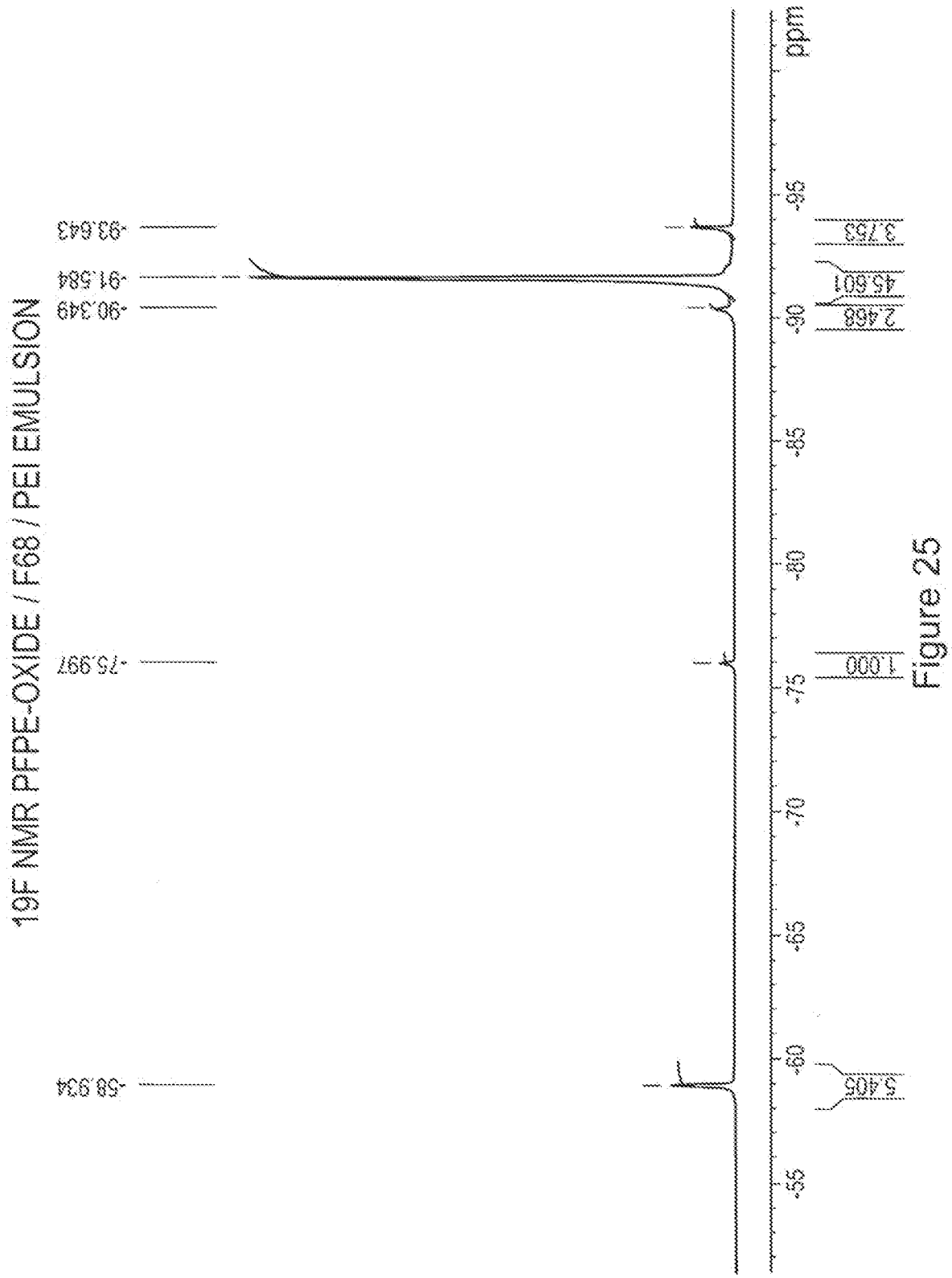

19F NMR PFPE-OXIDE / F68 / PEI EMULSION
-58.934
-75.997
-90.349
-91.584
-93.643
ppm
-55
-60
-65
-70
-75
-80
-85
-90
-95
5.405
1.000
2.468
45.601
3.753

Figure 25

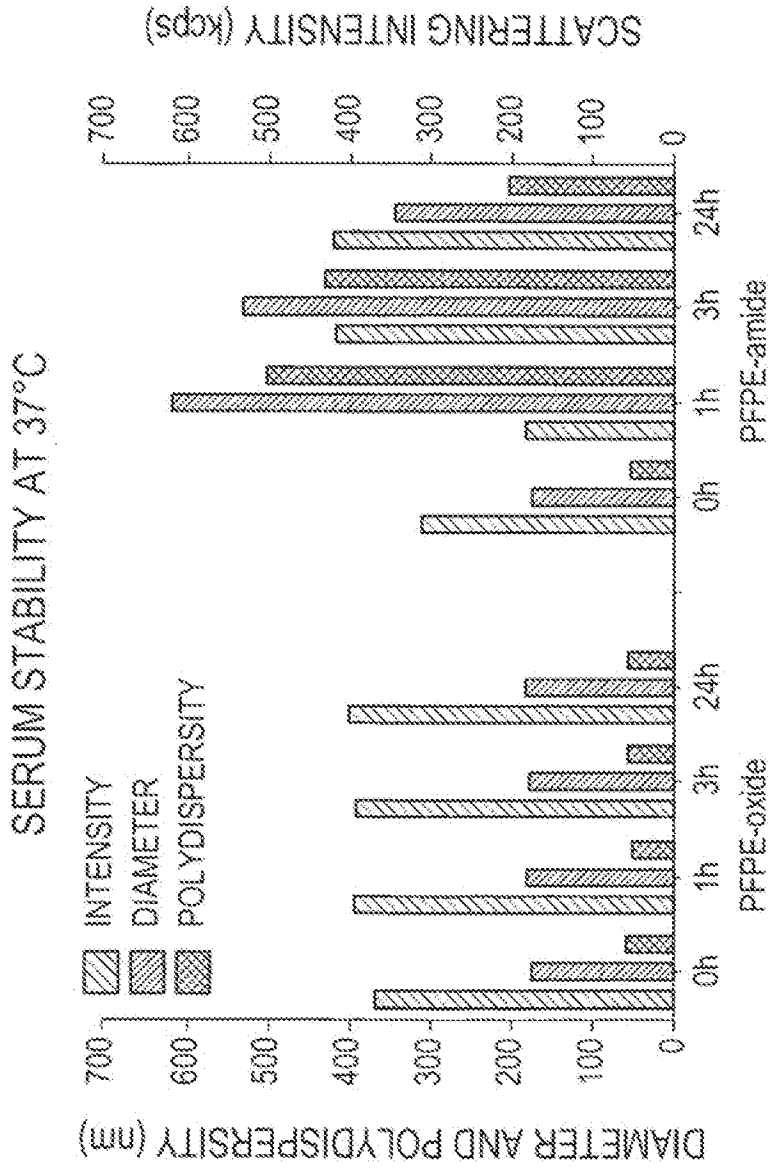

SERUM STABILITY AT 37°C
INTENSITY
DIAMETER
POLYDISPERSITY
DIAMETER AND POLYDISPERSITY (nm)
SCATTERING INTENSITY (kcps)
0
100
200
300
400
500
600
700
0h
1h
3h
24h
PFPE-oxide
PFPE-amide

Figure 26

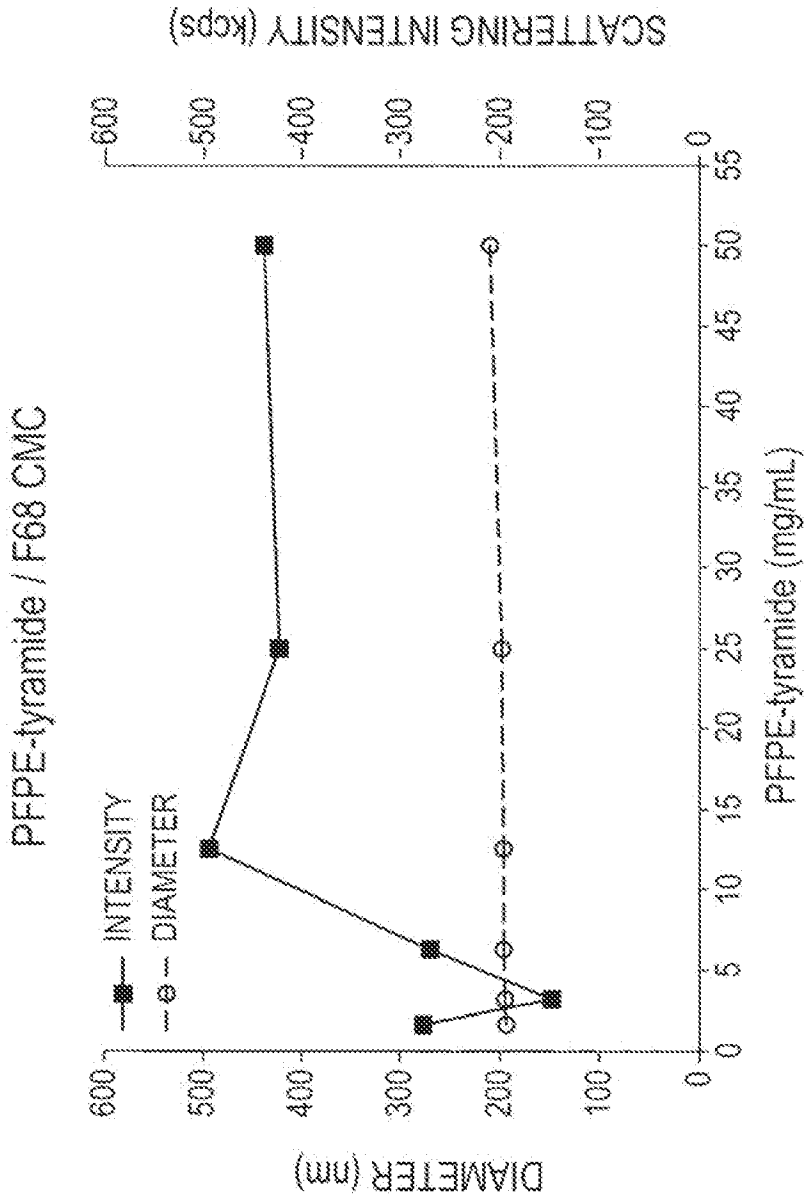

PFPE-tyramide / F68 CMC
INTENSITY
DIAMETER
DIAMETER (nm)
SCATTERING INTENSITY (kcps)
PFPE-tyramide (mg/mL)
600
500
400
300
200
100
0
5
10
15
20
25
30
35
40
45
50
55

Figure 27

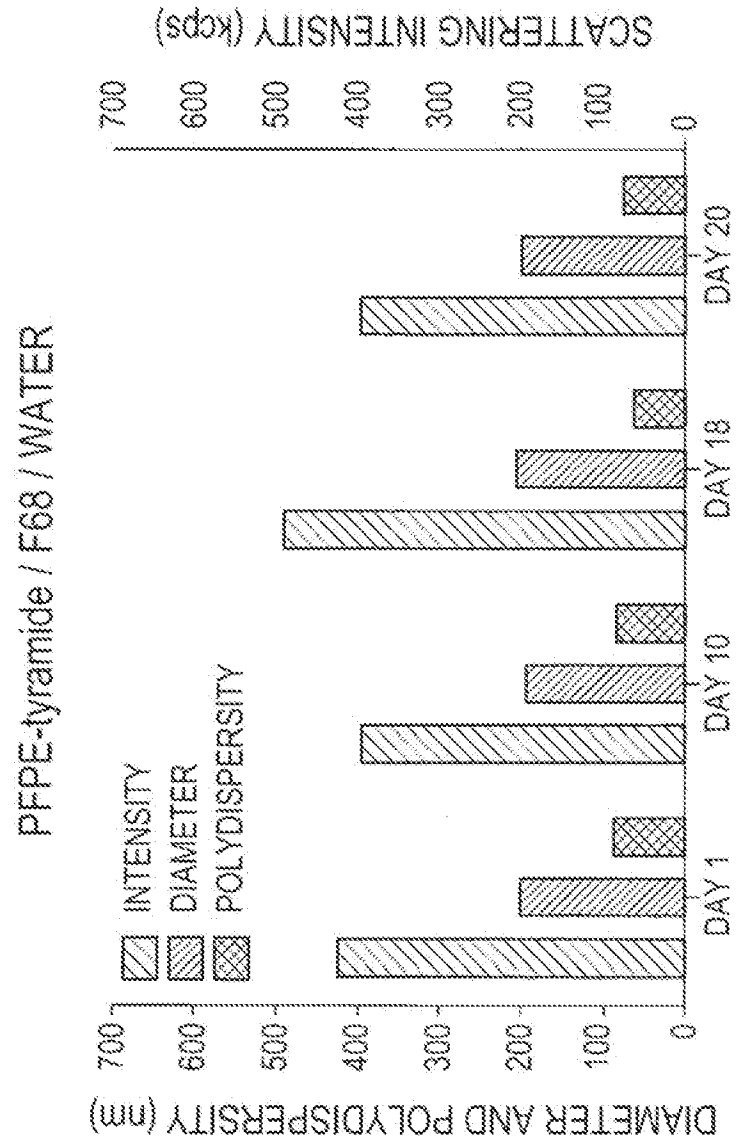

PFPE-tyramide / F68 / WATER
INTENSITY
DIAMETER
POLYDISPERSITY
DIAMETER AND POLYDISPERSITY (nm)
SCATTERING INTENSITY (kcps)
0
100
200
300
400
500
600
700
DAY 1
DAY 10
DAY 18
DAY 20

Figure 28

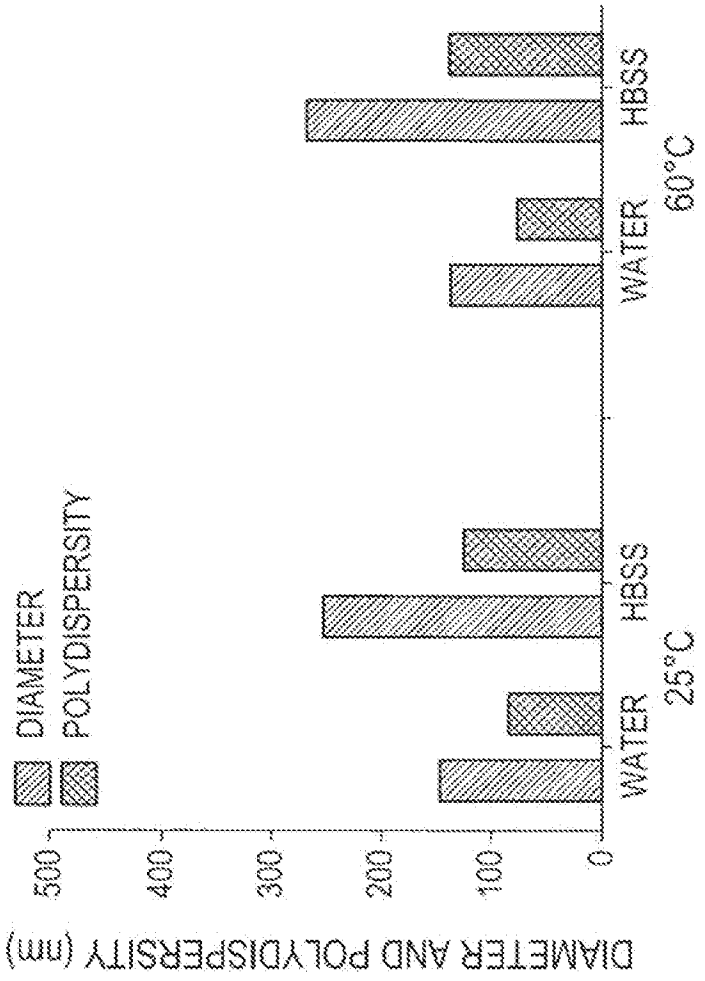

PFPE-NH(EtO)2OH / F68
DIAMETER
POLYDISPERSITY
DIAMETER AND POLYDISPERSITY (nm)
500
400
300
200
100
0
WATER
HBSS
WATER
HBSS
25°C
60°C

Figure 29

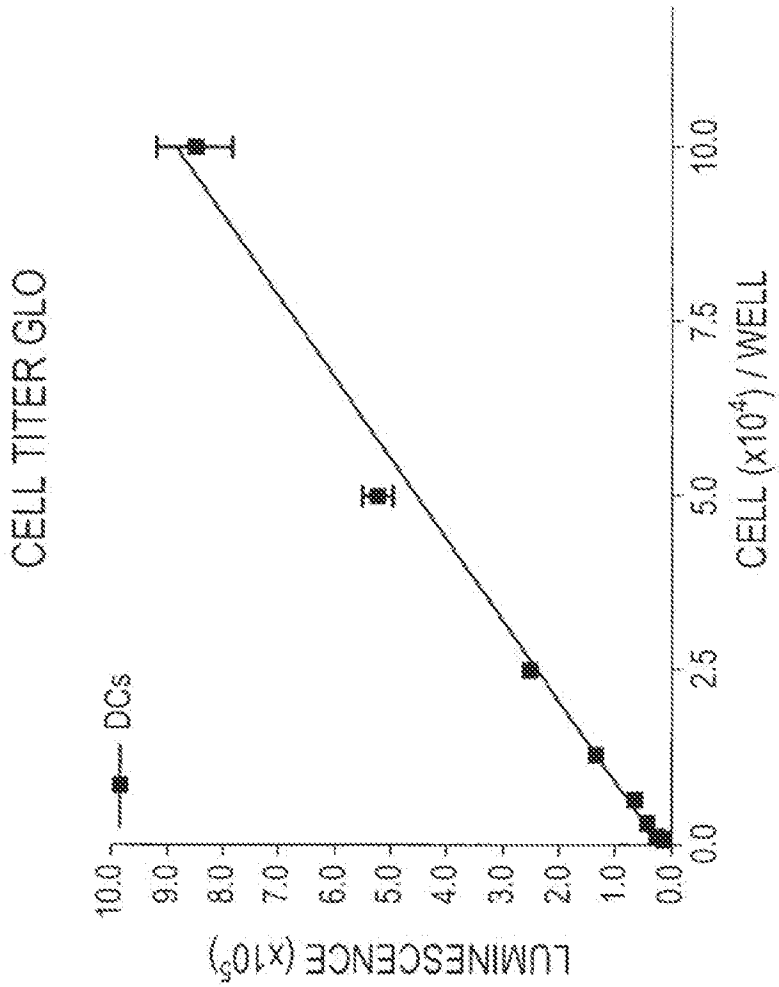

CELL TITER GLO
DCs
LUMINESCENCE (x10^5)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.0
2.5
5.0
7.5
10.0
CELL (x10^4) / WELL

Figure 30

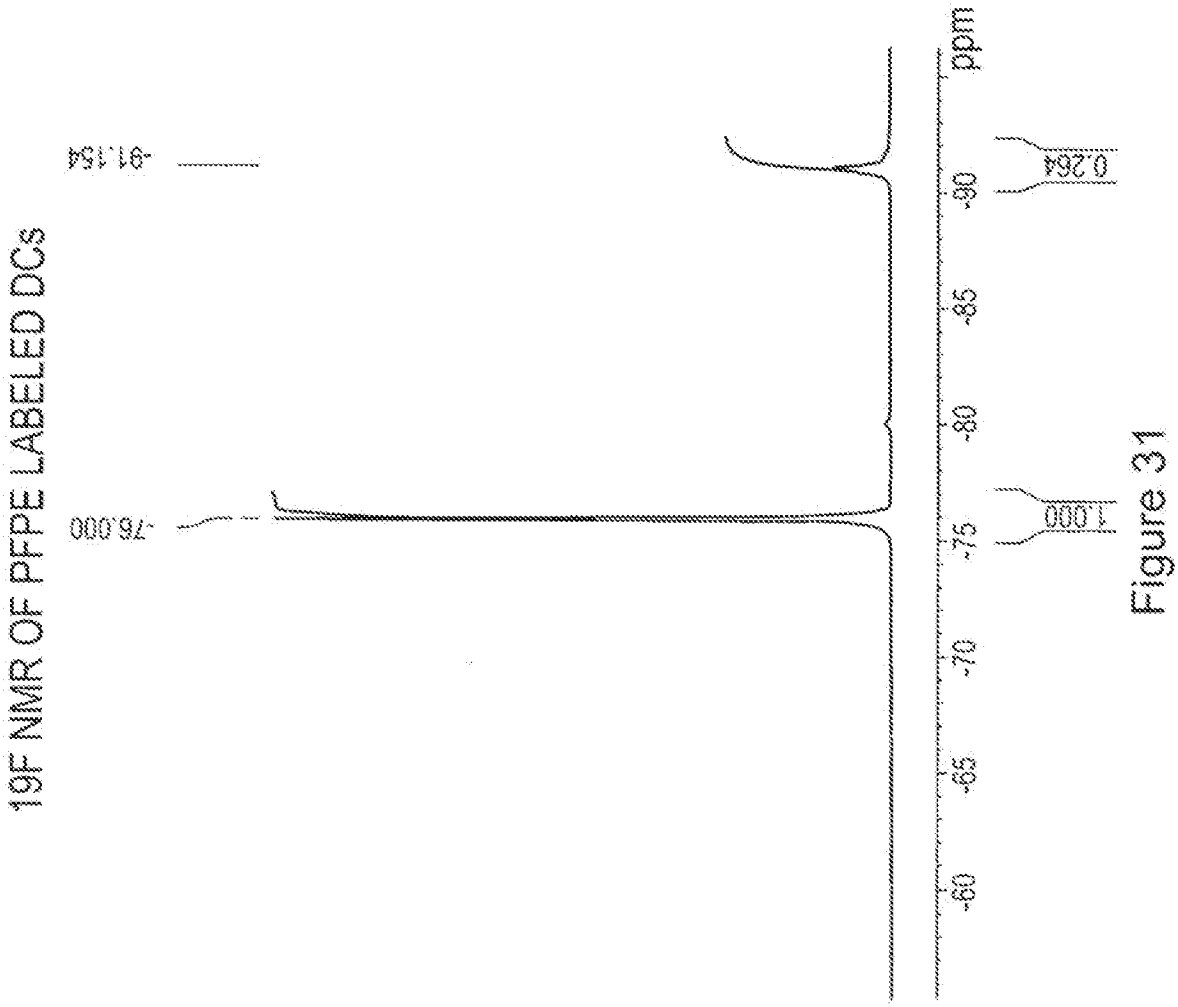

19F NMR OF PFPE LABELED DCs
-91.154
-76.000
ppm
-60
-65
-70
-75
-80
-85
-90
1.000
0.264

Figure 31

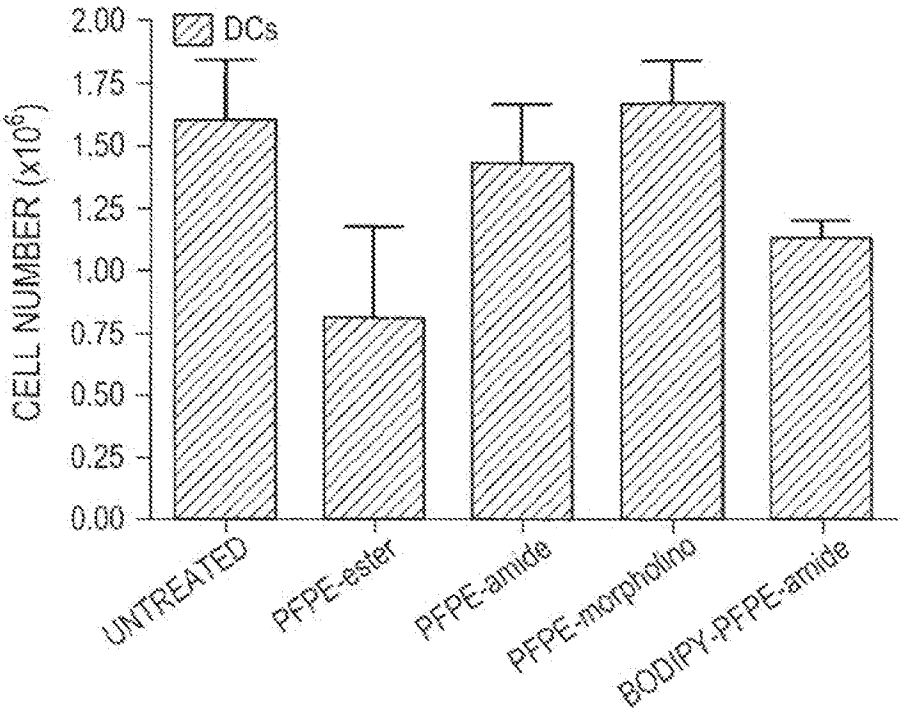


Figure 32A

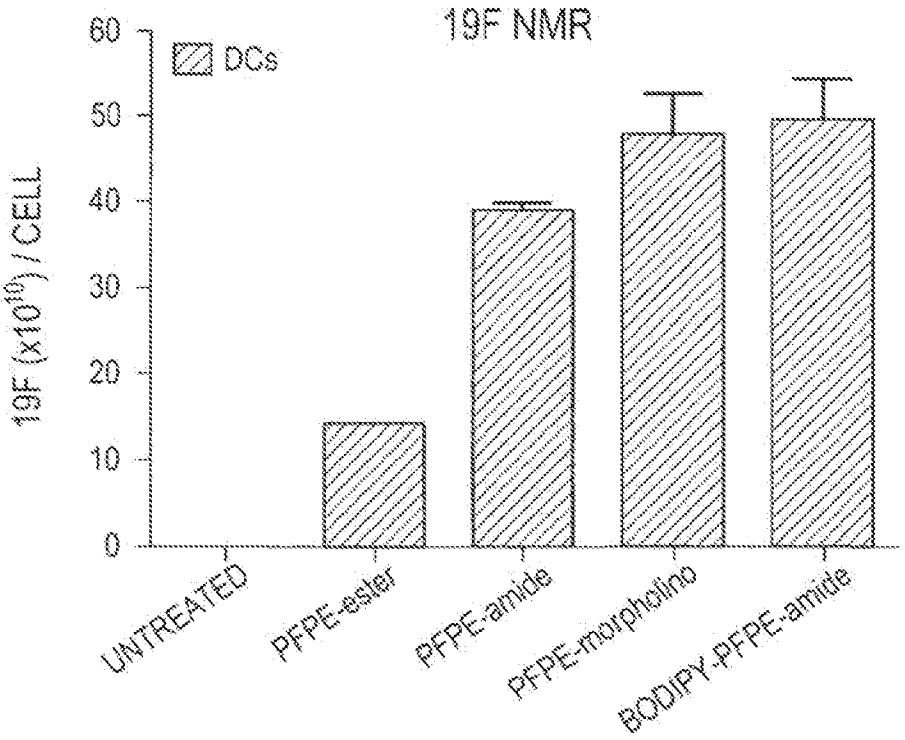


Figure 32B

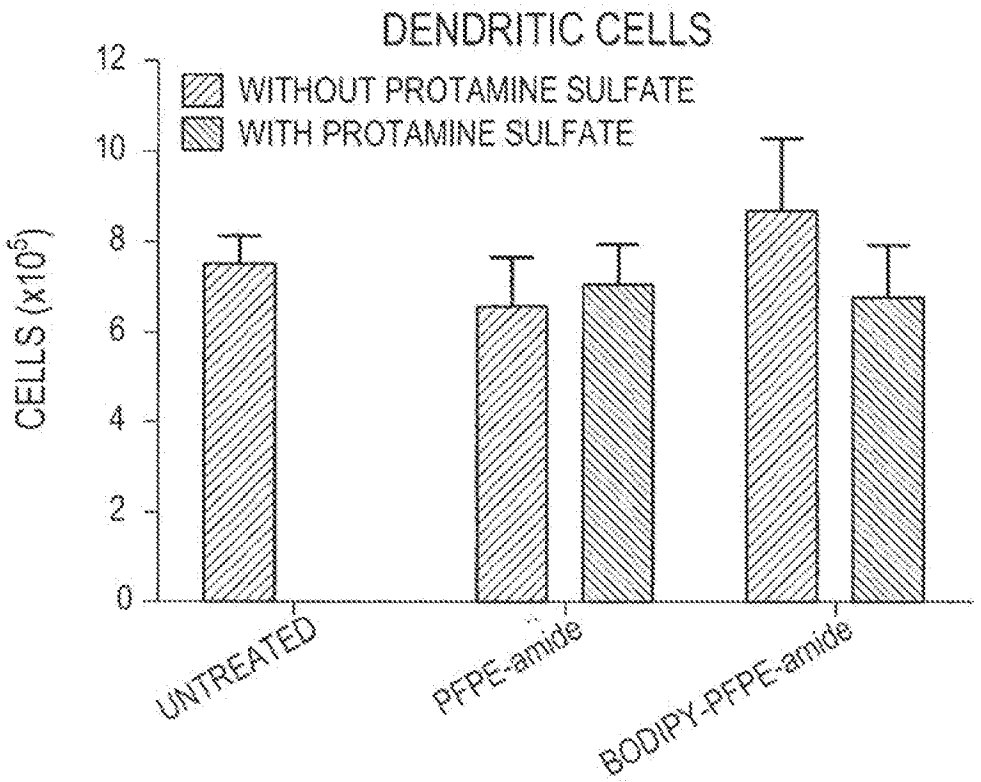
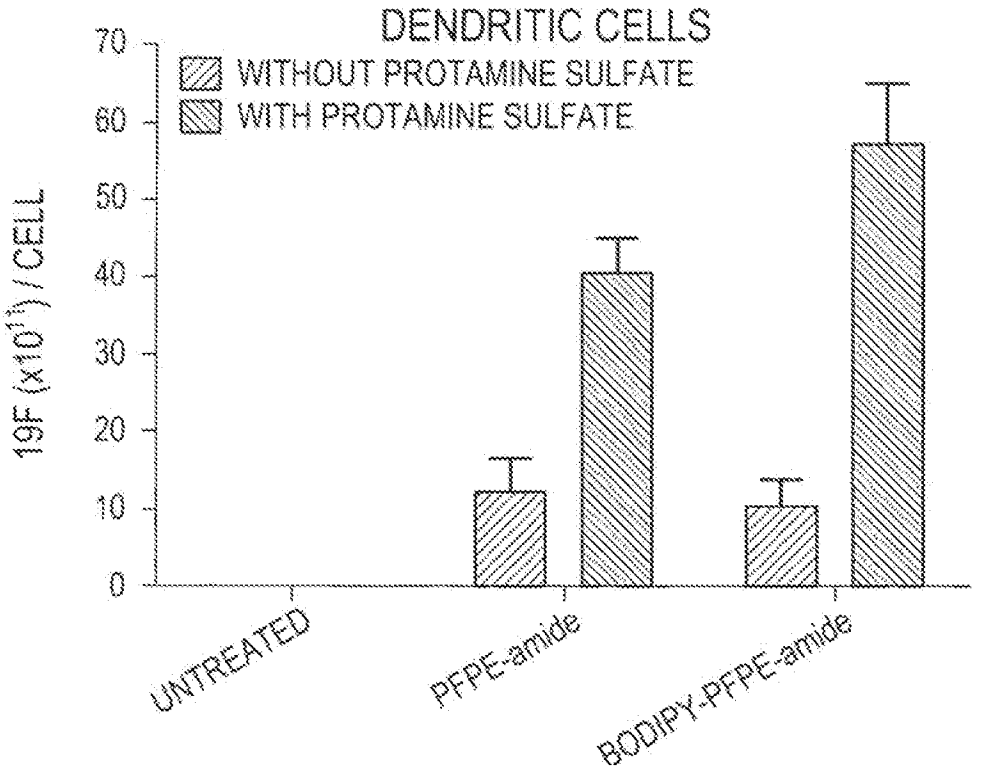

DENDRITIC CELLS
WITHOUT PROTAMINE SULFATE
WITH PROTAMINE SULFATE
CELLS (x10^5)
12
10
8
6
4
2
0
UNTREATED
PFPE-amide
BODIPY-PFPE-amide

Figure 33A


DENDRITIC CELLS
WITHOUT PROTAMINE SULFATE
WITH PROTAMINE SULFATE
19F (x10^1) / CELL
70
60
50
40
30
20
10
0
UNTREATED
PFPE-amide
BODIPY-PFPE-amide

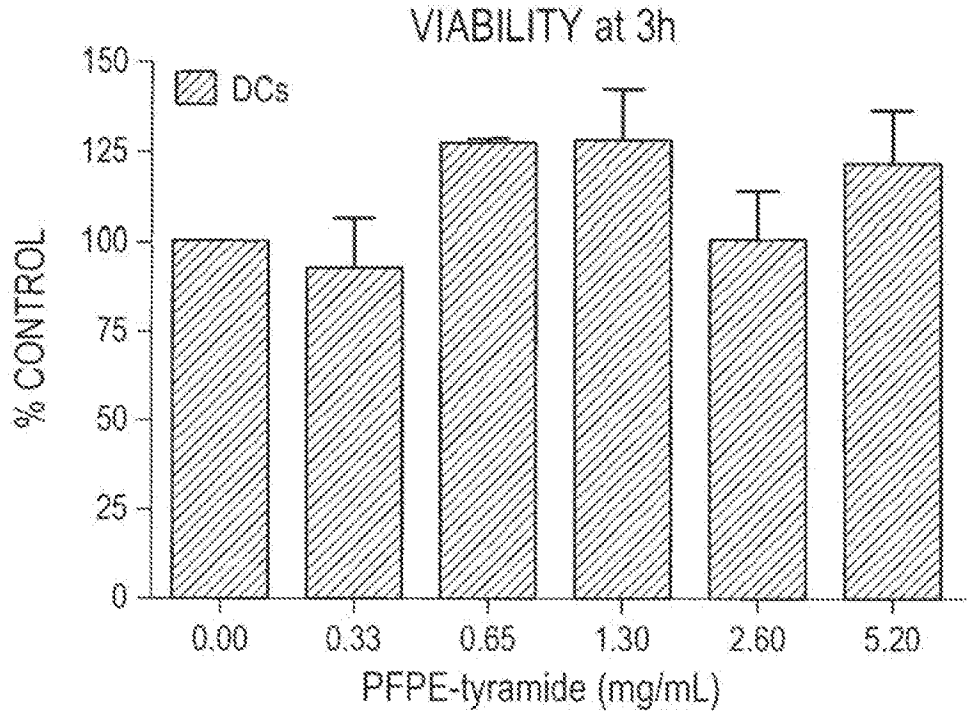
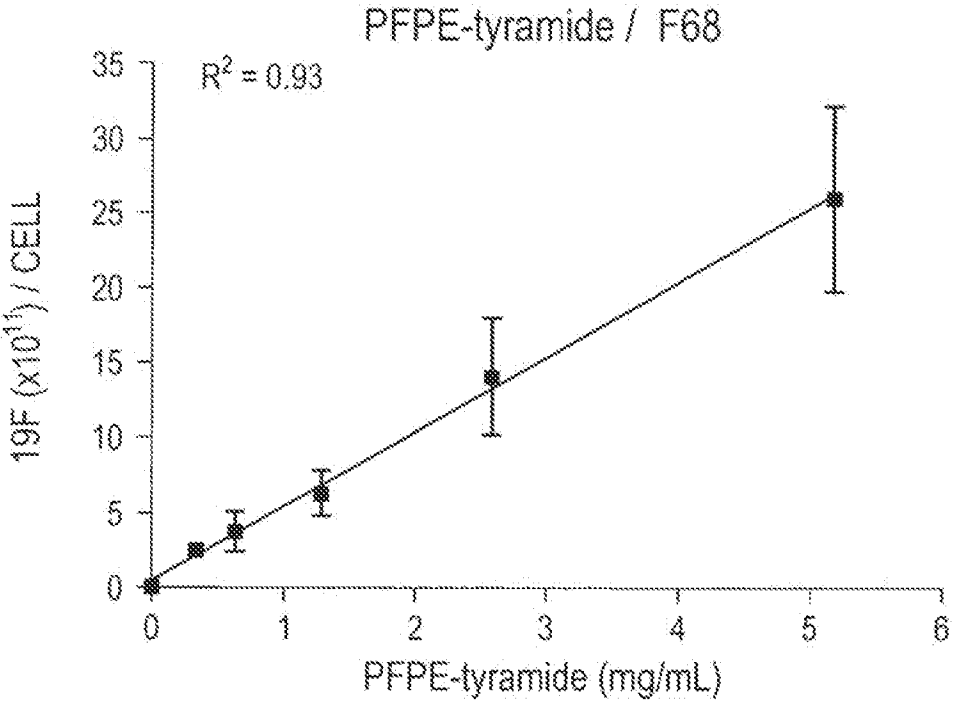

VIABILITY at 3h
DCs
% CONTROL
150
125
100
75
50
25
0
0.00
0.33
0.65
1.30
2.60
5.20
PFPE-tyramide (mg/mL)

Figure 34A


PFPE-tyramide / F68
R2 = 0.93
19F (x10^11) / CELL
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
PFPE-tyramide (mg/mL)

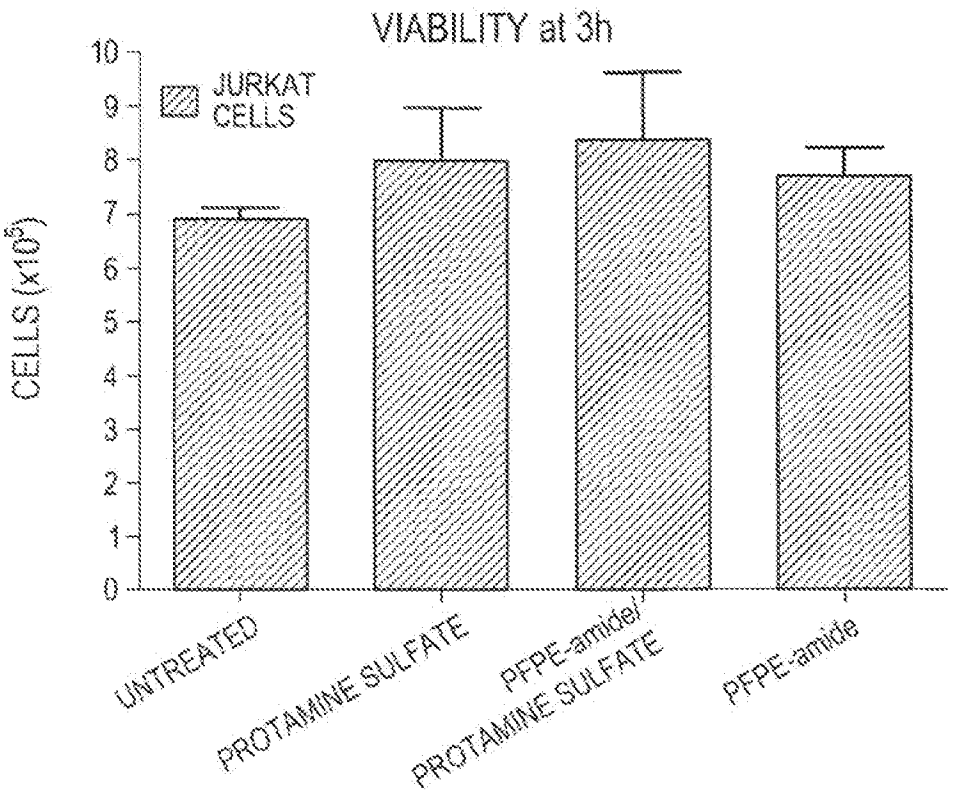


Figure 35A

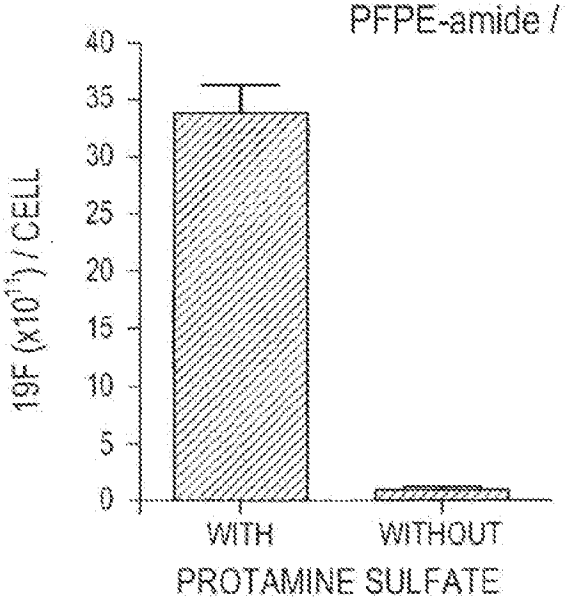


Figure 35B

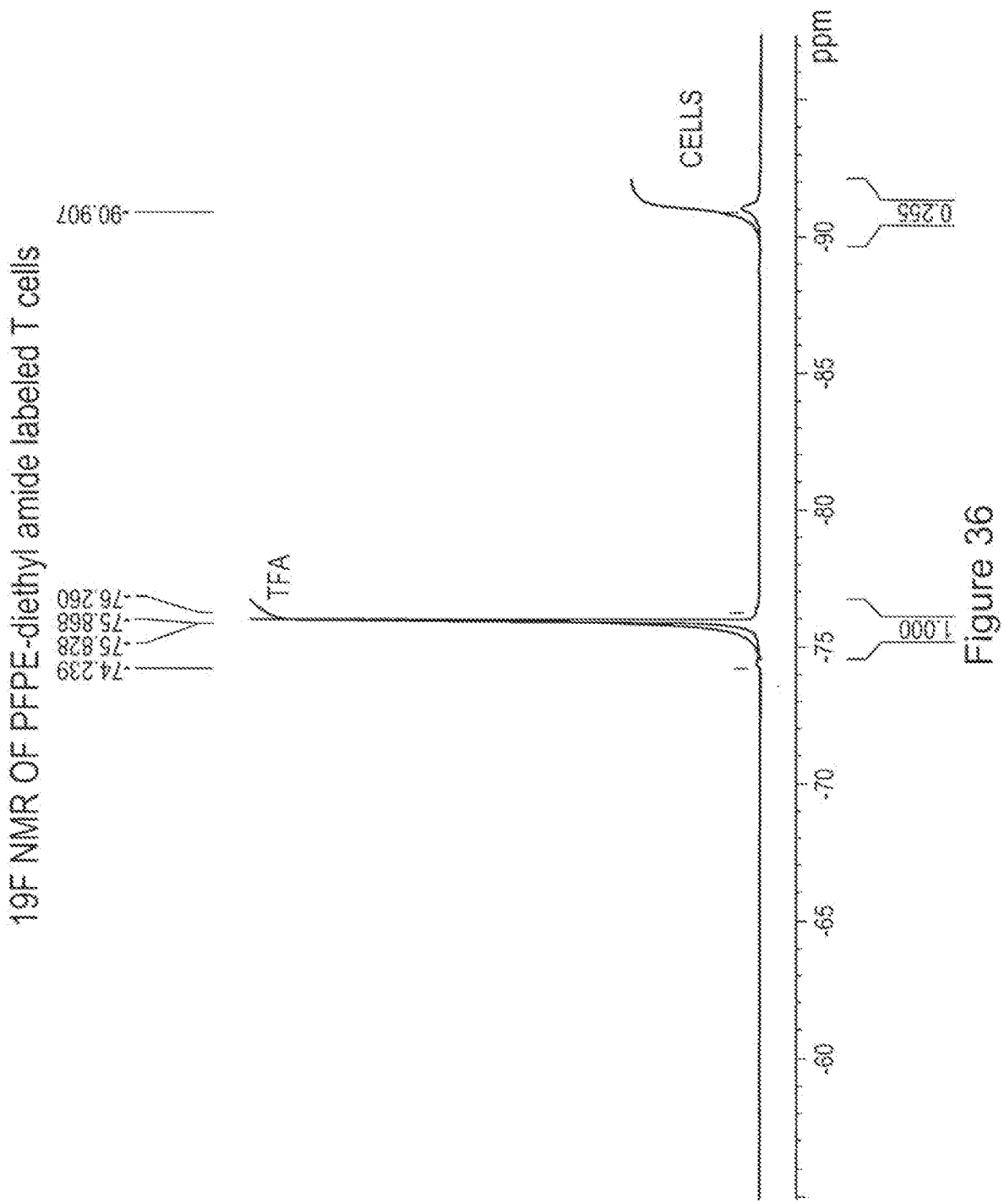

19F NMR OF PFPE-diethyl amide labeled T cells
-90.907
-74.239
-75.828
-75.868
-76.260
TFA
CELLS
ppm
-60
-65
-70
-75
-80
-85
-90
1.000
0.265

Figure 36

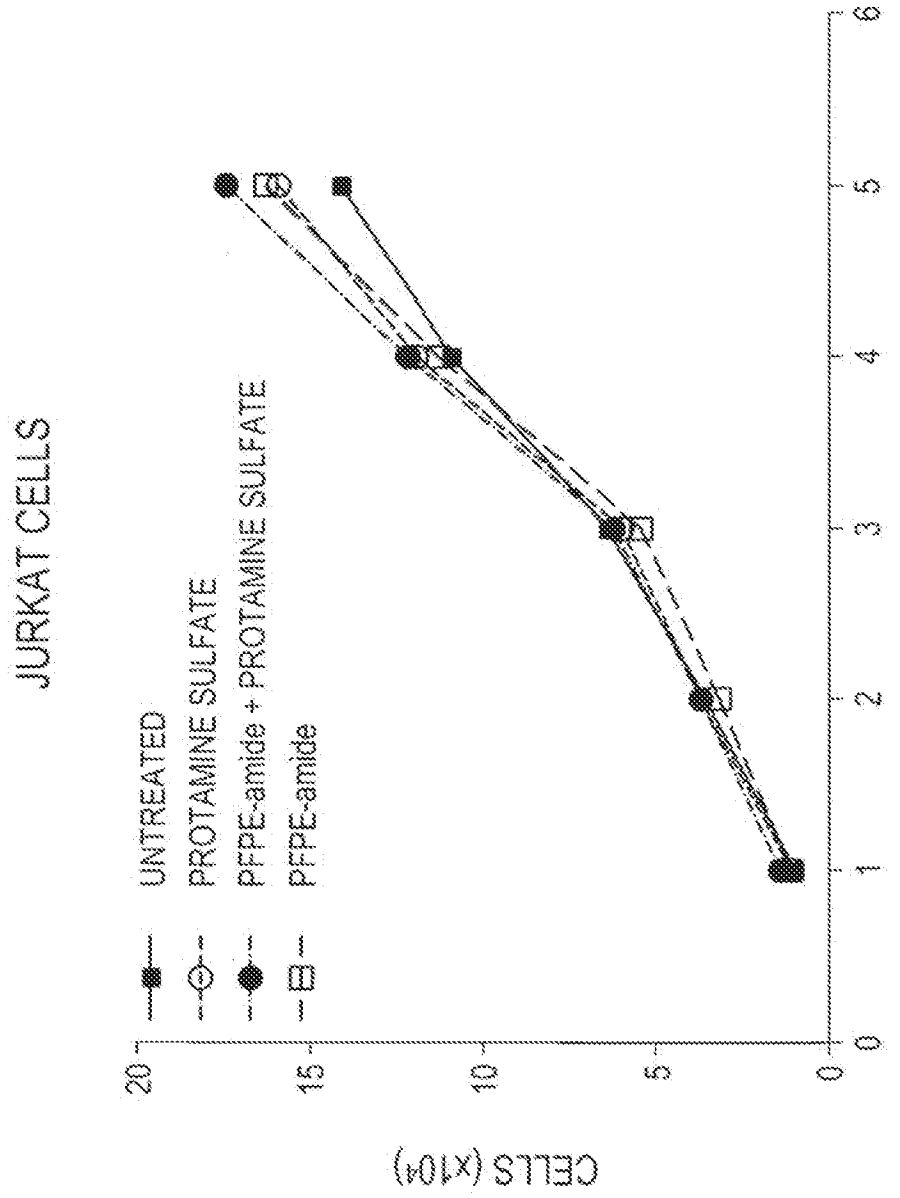

JURKAT CELLS
UNTREATED
PROTAMINE SULFATE
PFPE-amide + PROTAMINE SULFATE
PFPE-amide
CELLS (x10⁴)
DAYS
0
5
10
15
20
0
1
2
3
4
5
6

Figure 37

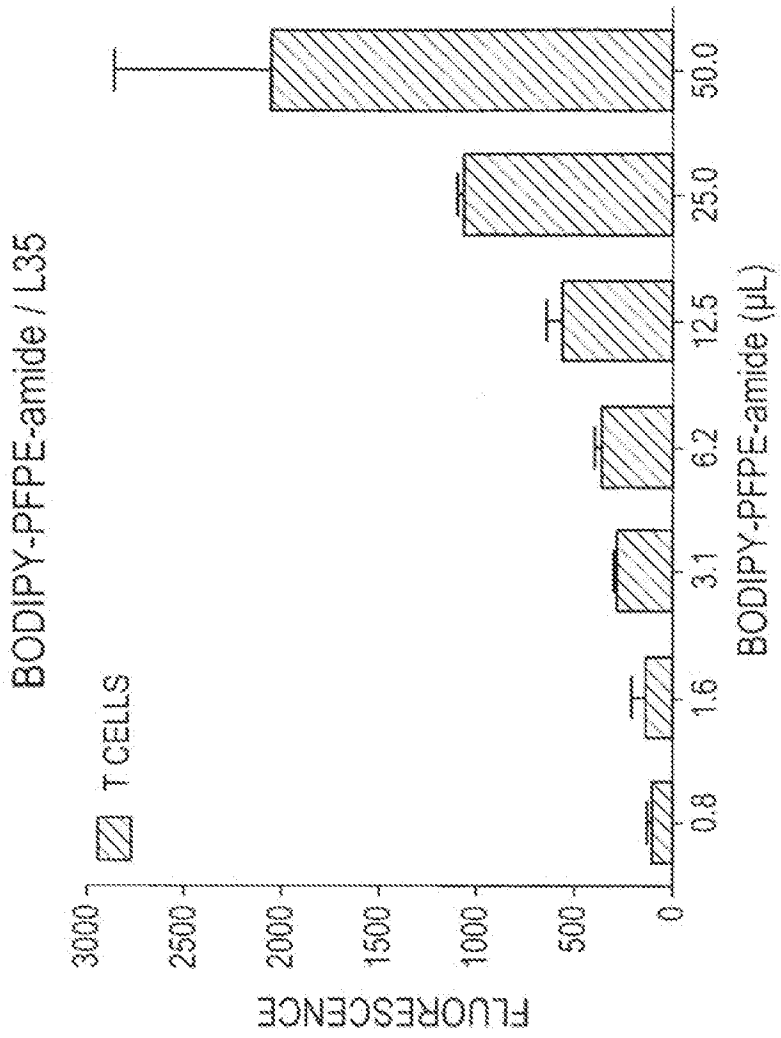

BODIPY-PFPE-amide / L35
T CELLS
FLUORESCENCE
3000
2500
2000
1500
1000
500
0
0.8
1.6
3.1
6.2
12.5
25.0
50.0
BODIPY-PFPE-amide (µL)

Figure 38

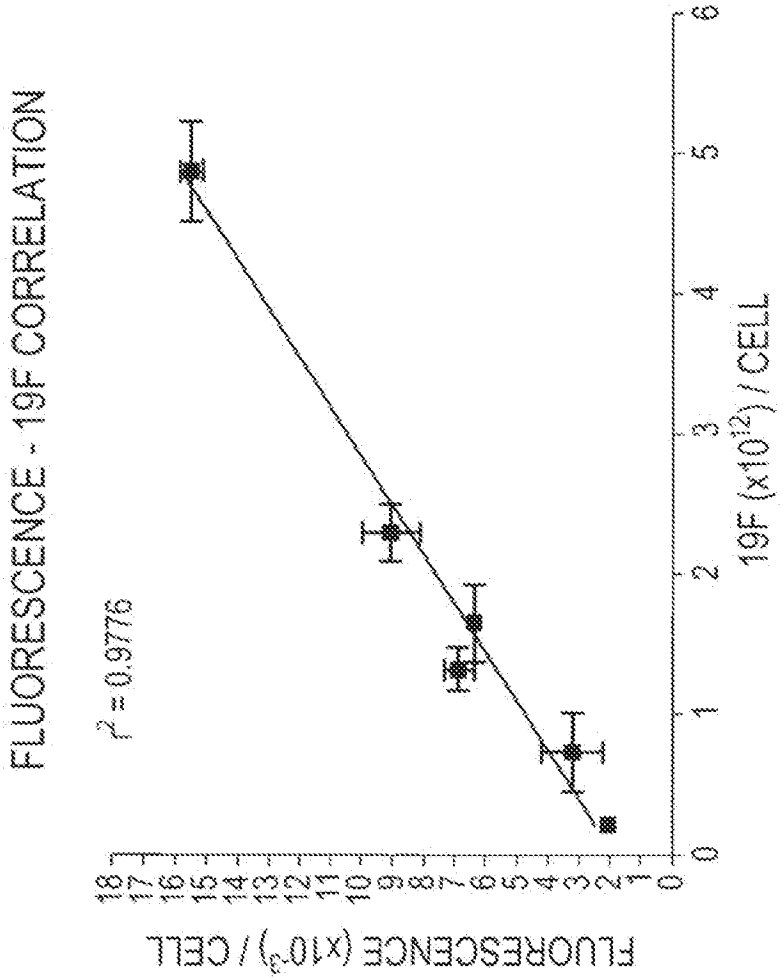

FLUORESCENCE - 19F CORRELATION
$r^2 = 0.9776$
FLUORESCENCE ($x10^3$) / CELL
19F ($x10^{12}$) / CELL
0
1
2
3
4
5
6
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Figure 39

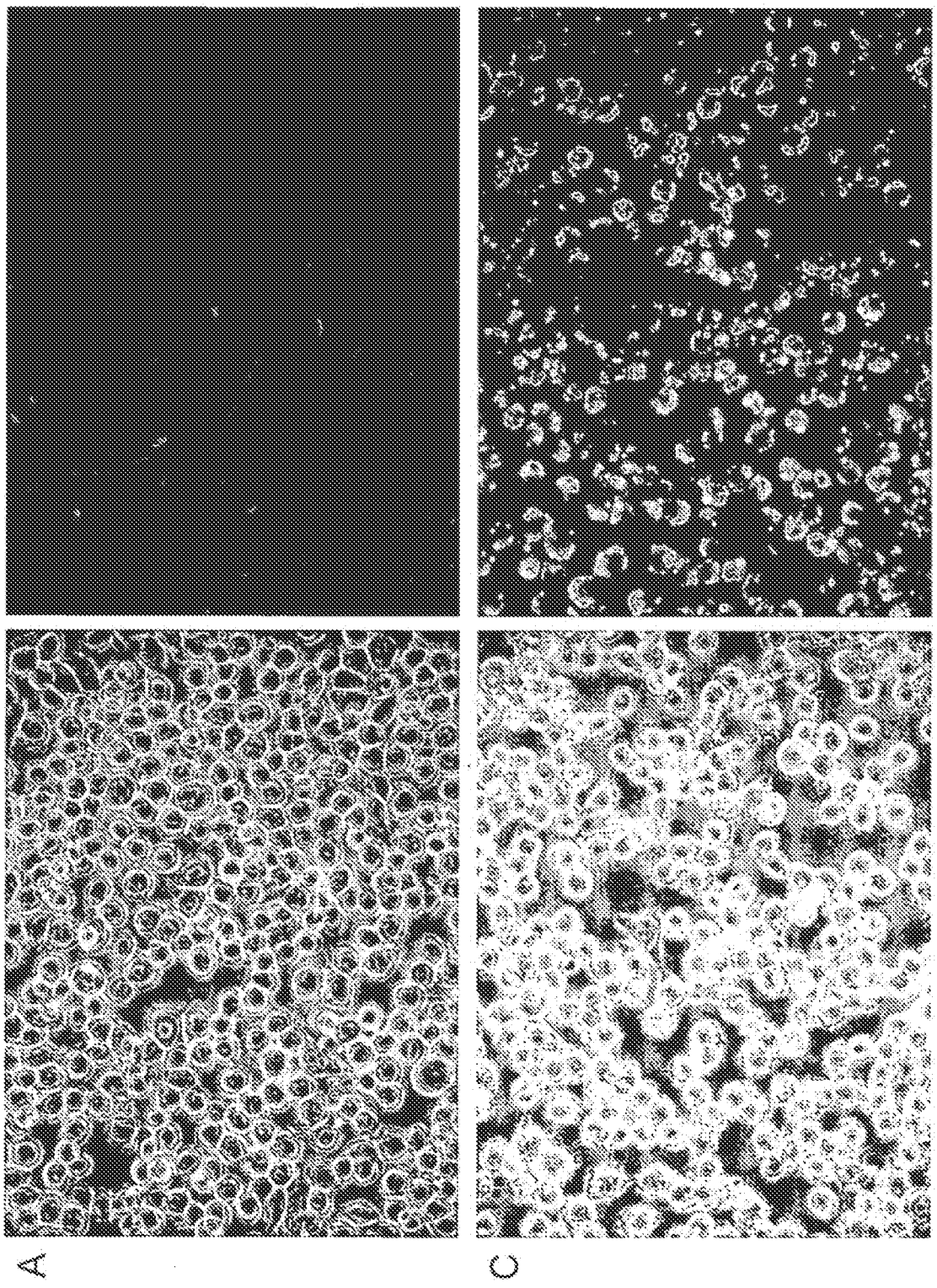


Figure 40

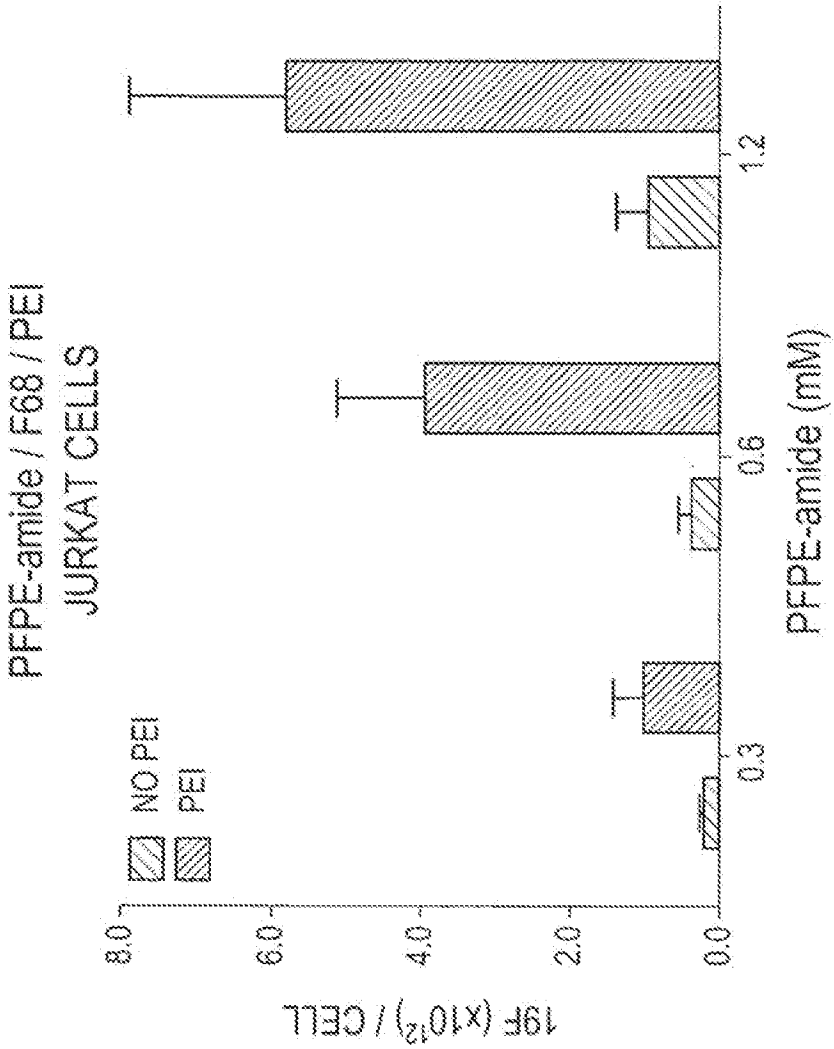

PFPE-amide / F68 / PEI
JURKAT CELLS
NO PEI
PEI
19F (x10^12) / CELL
8.0
6.0
4.0
2.0
0.0
0.3
0.6
1.2
PFPE-amide (mM)

Figure 41

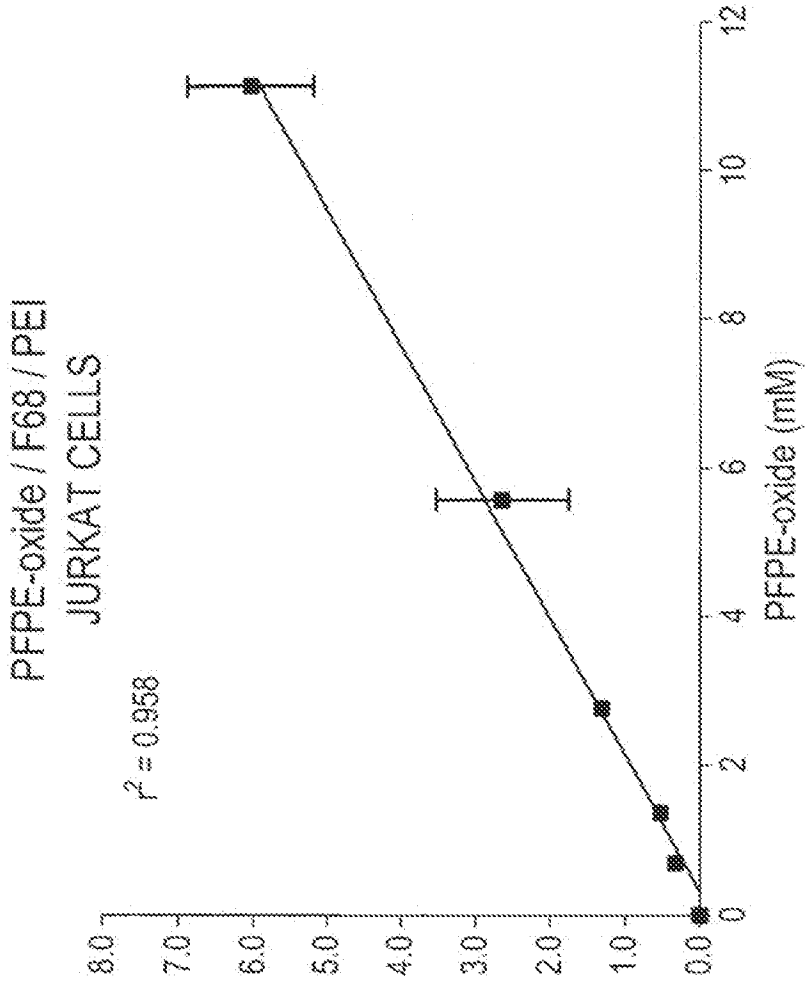

PFPE-oxide / F68 / PEI
JURKAT CELLS
$r^2 = 0.958$
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
2
4
6
8
10
12
PFPE-oxide (mM)

Figure 42

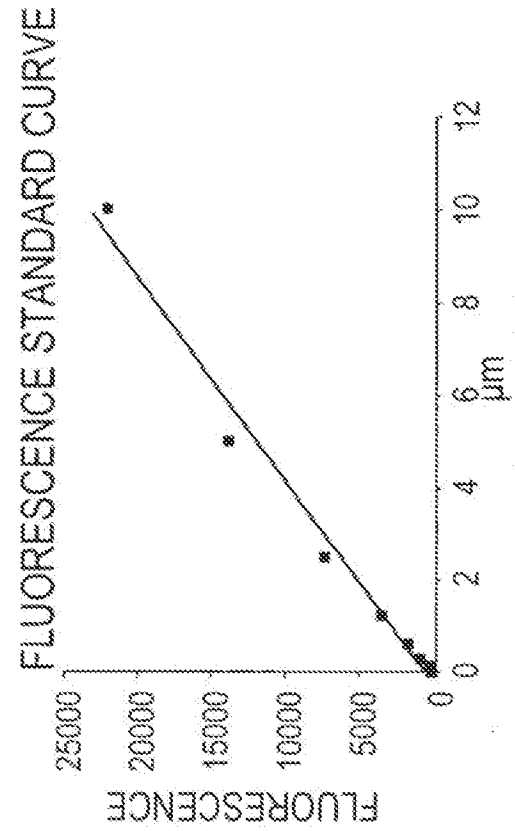


Figure 43A

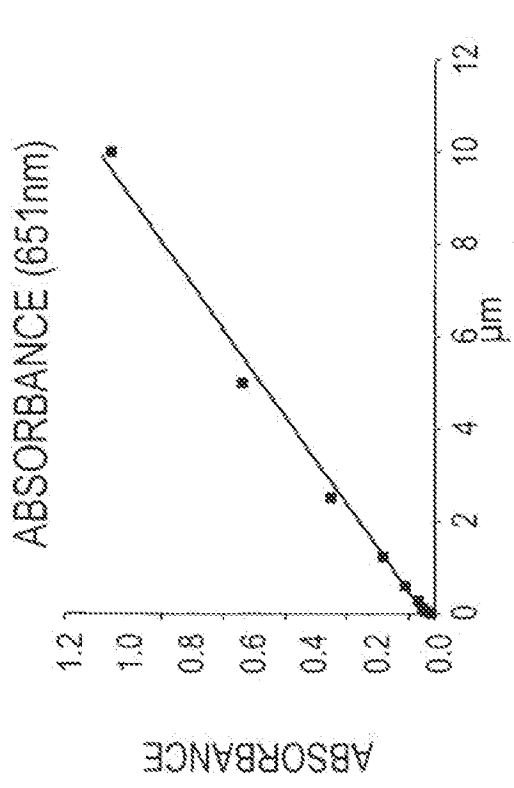


Figure 43B

Figure 43C

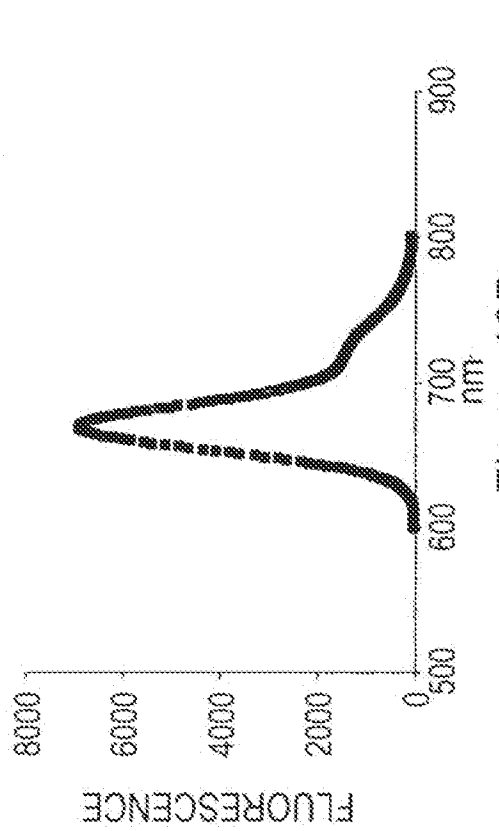


Figure 43D

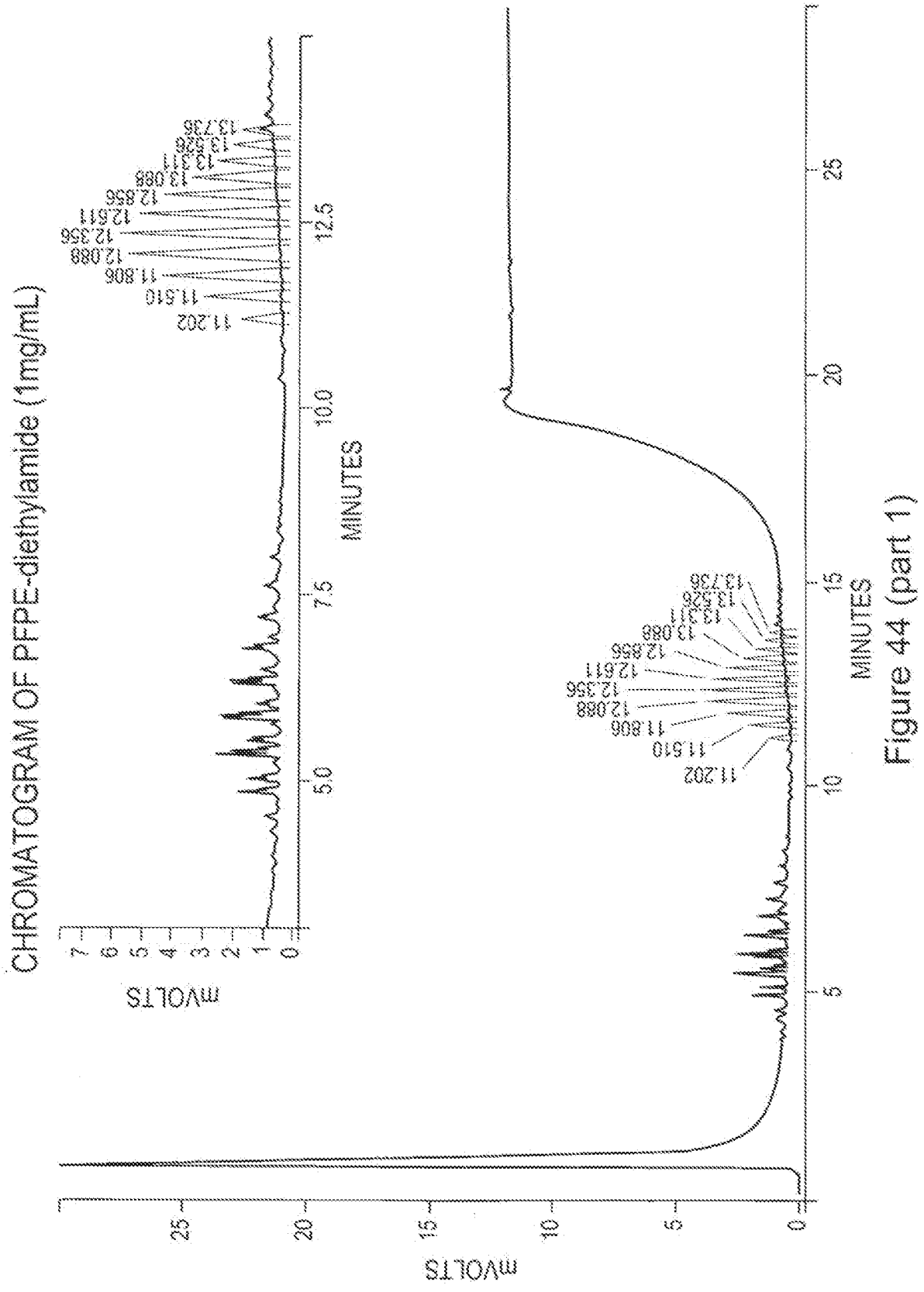


Figure 44 (part 1)

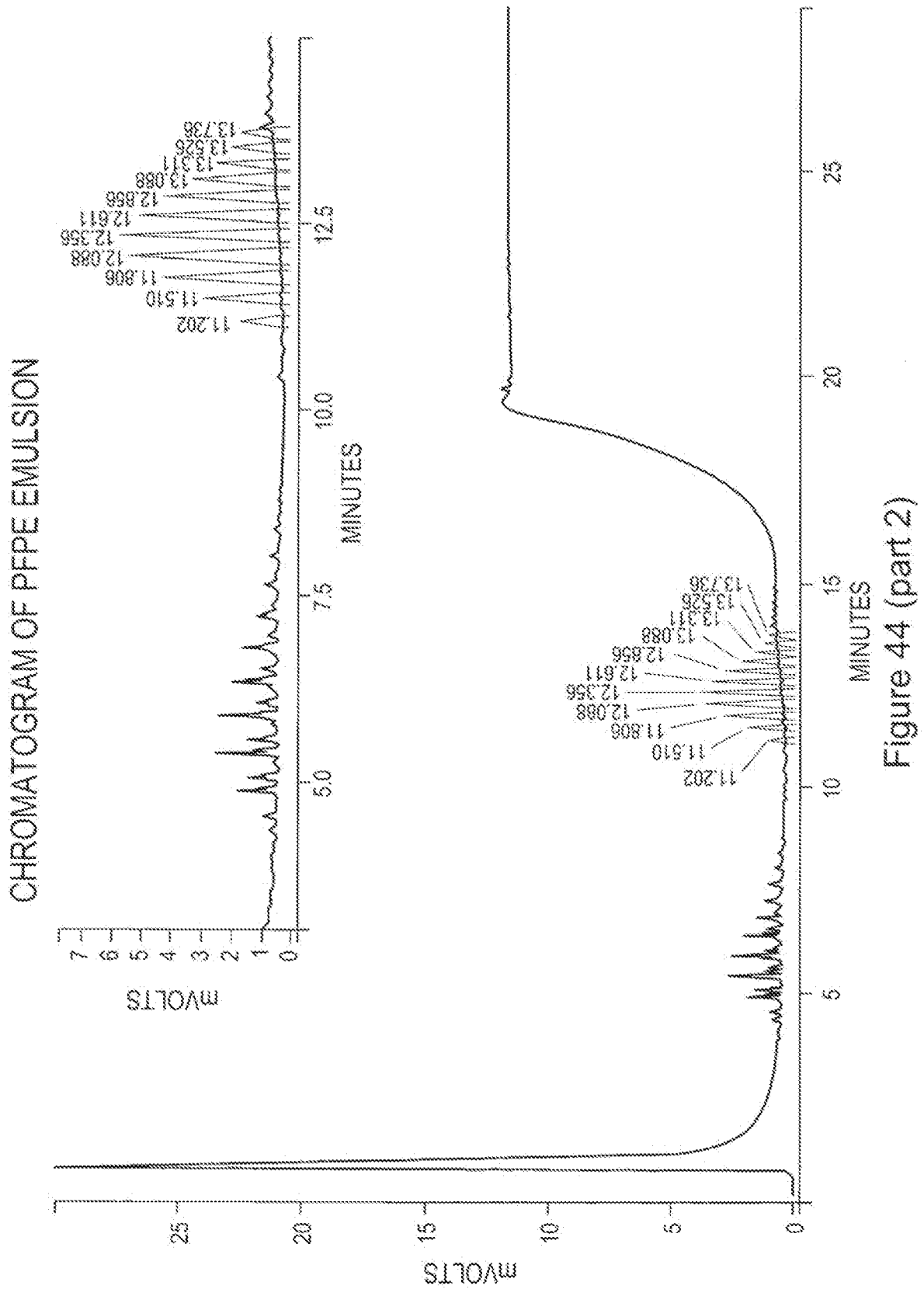


Figure 44 (part 2)

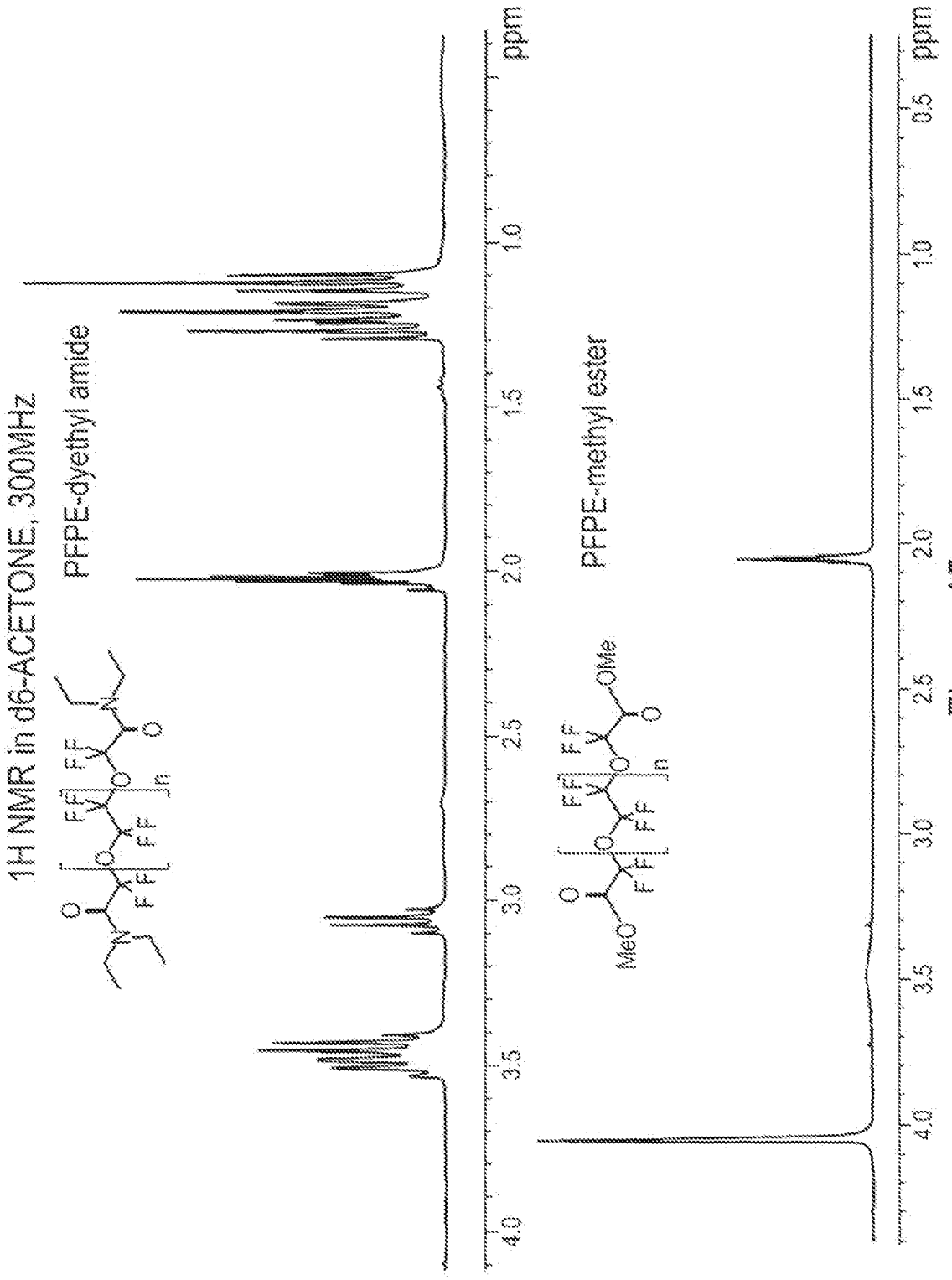

1H NMR in d6-ACETONE, 300MHz
PFPE-dyethyl amide
PFPE-methyl ester
ppm
ppm

Figure 45

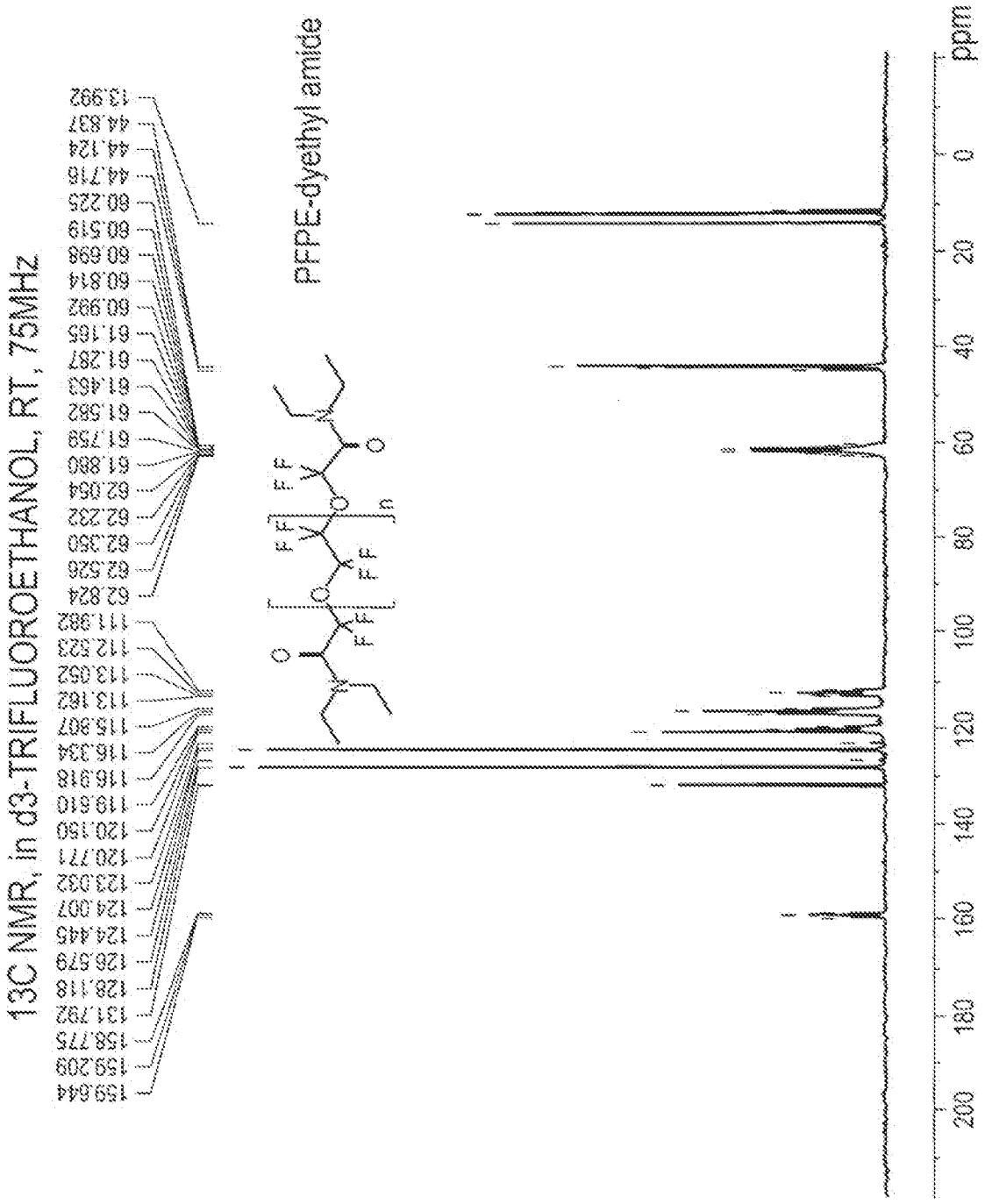

13C NMR, in d3-TRIFLUOROETHANOL, RT, 75MHz
PFPE-dyethyl amide
ppm
0
20
40
60
80
100
120
140
160
180
200

Figure 46

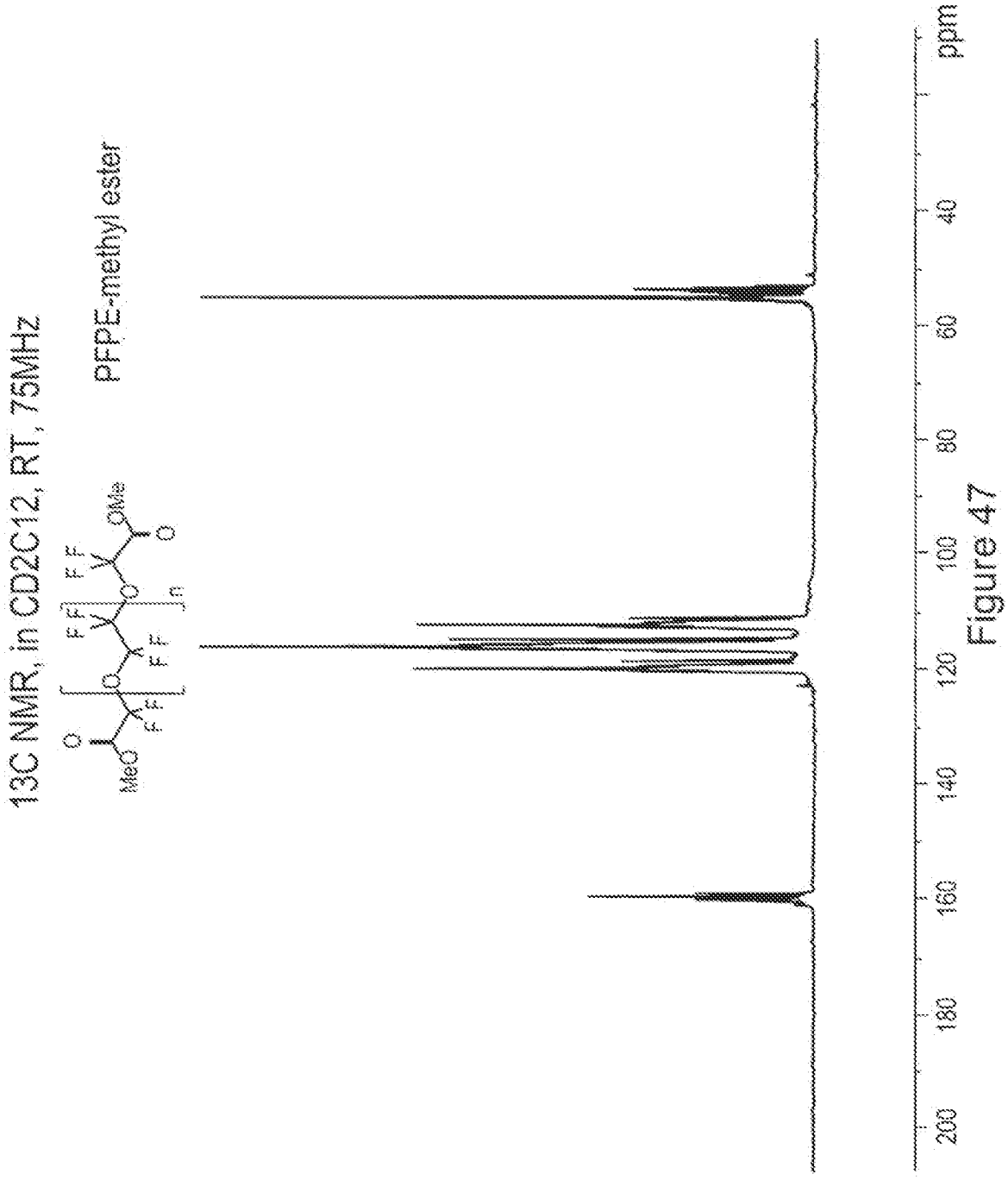

13C NMR, in CD2C12, RT, 75MHz
PFPE-methyl ester
MeO
OMe
n
200
180
160
140
120
100
80
60
40
ppm

Figure 47

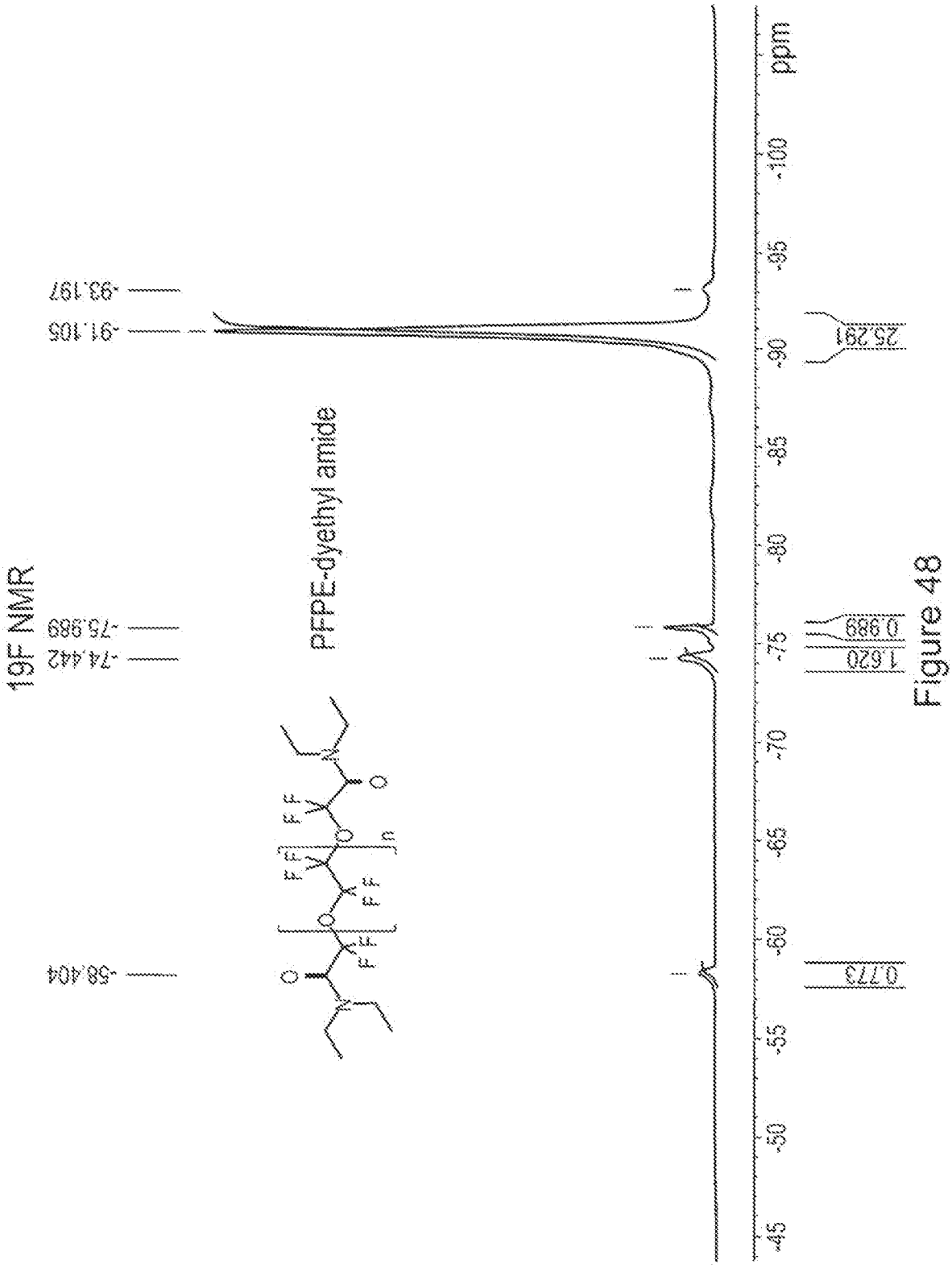

19F NMR
PFPE-diethyl amide
-58.404
-74.442
-75.989
-91.105
-93.197
25.291
1.620
0.989
0.773
ppm
-45
-50
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100

Figure 48

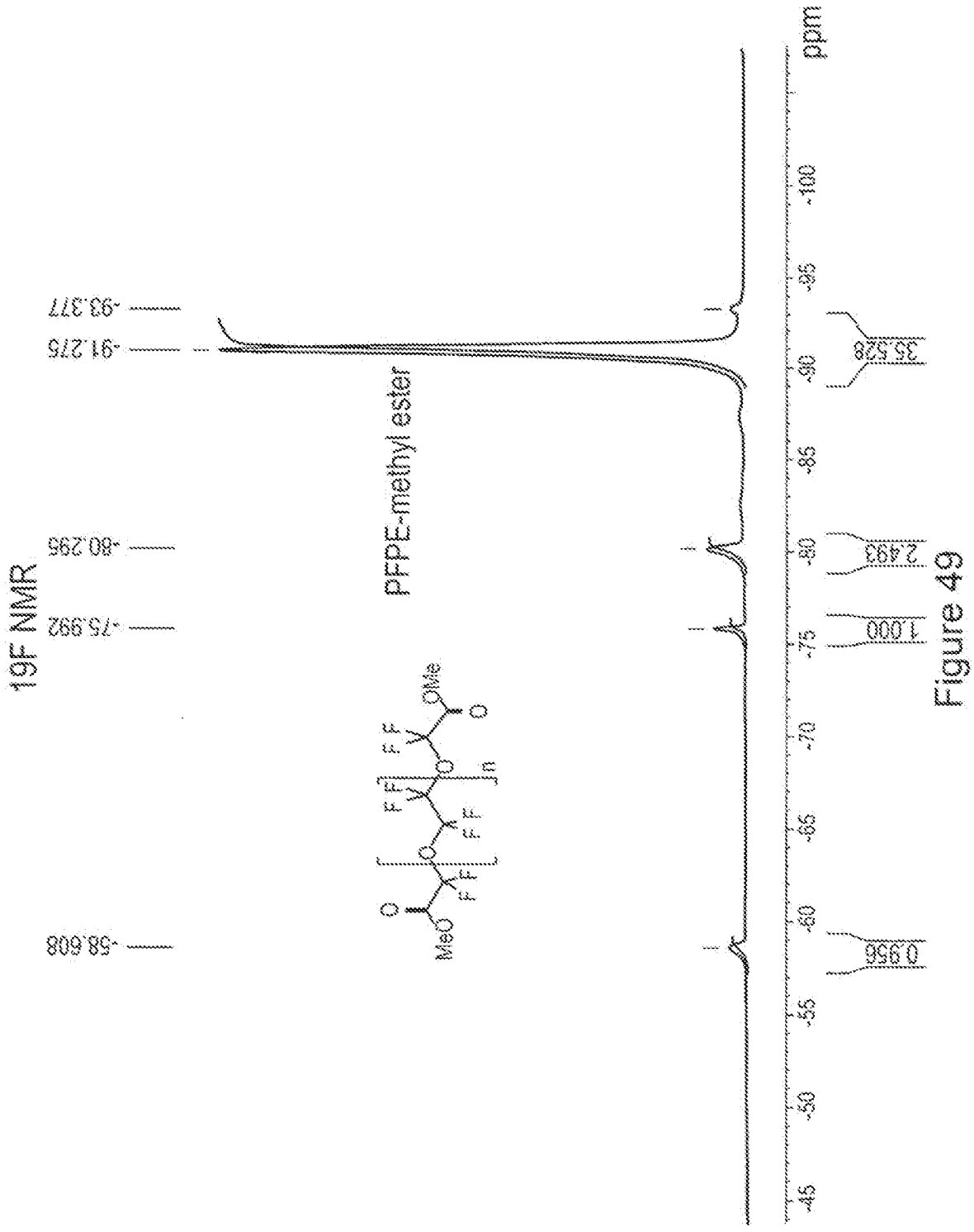

19F NMR
PFPE-methyl ester
-58.608
-75.992
-80.296
-91.275
-93.377
ppm
-45
-50
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
0.956
1.000
2.493
35.528
MeO
OMe
n

Figure 49

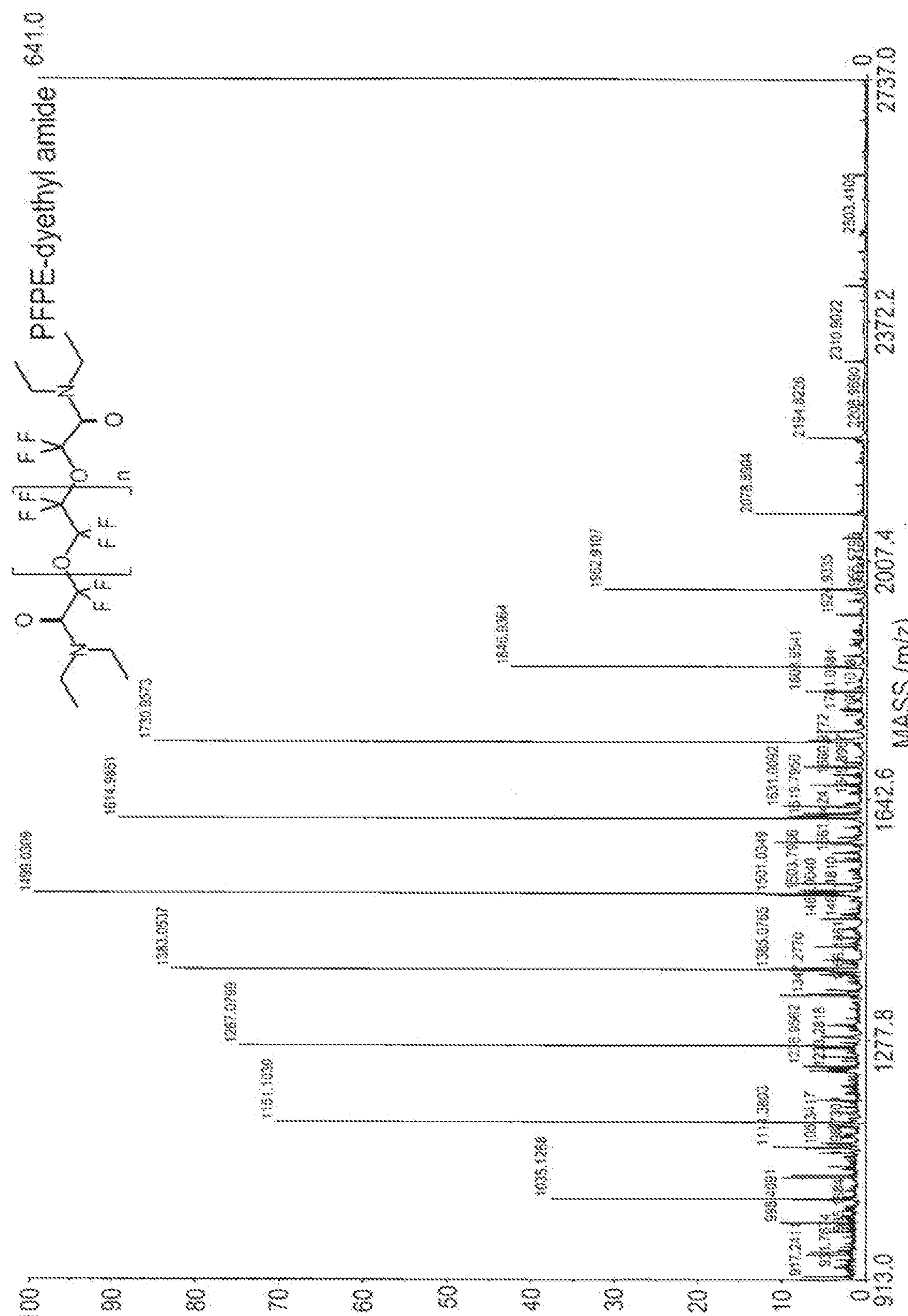

PFPE-dyethyl amide
MASS (m/z)
913.0
1277.8
1642.6
2007.4
2372.2
2737.0
0
10
20
30
40
50
60
70
80
90
100
641.0
1035.1268
1151.1030
1267.0759
1383.0537
1498.0309
1614.9851
1730.9573
1846.9364
1962.9107
2078.8884
2194.8236

Figure 50

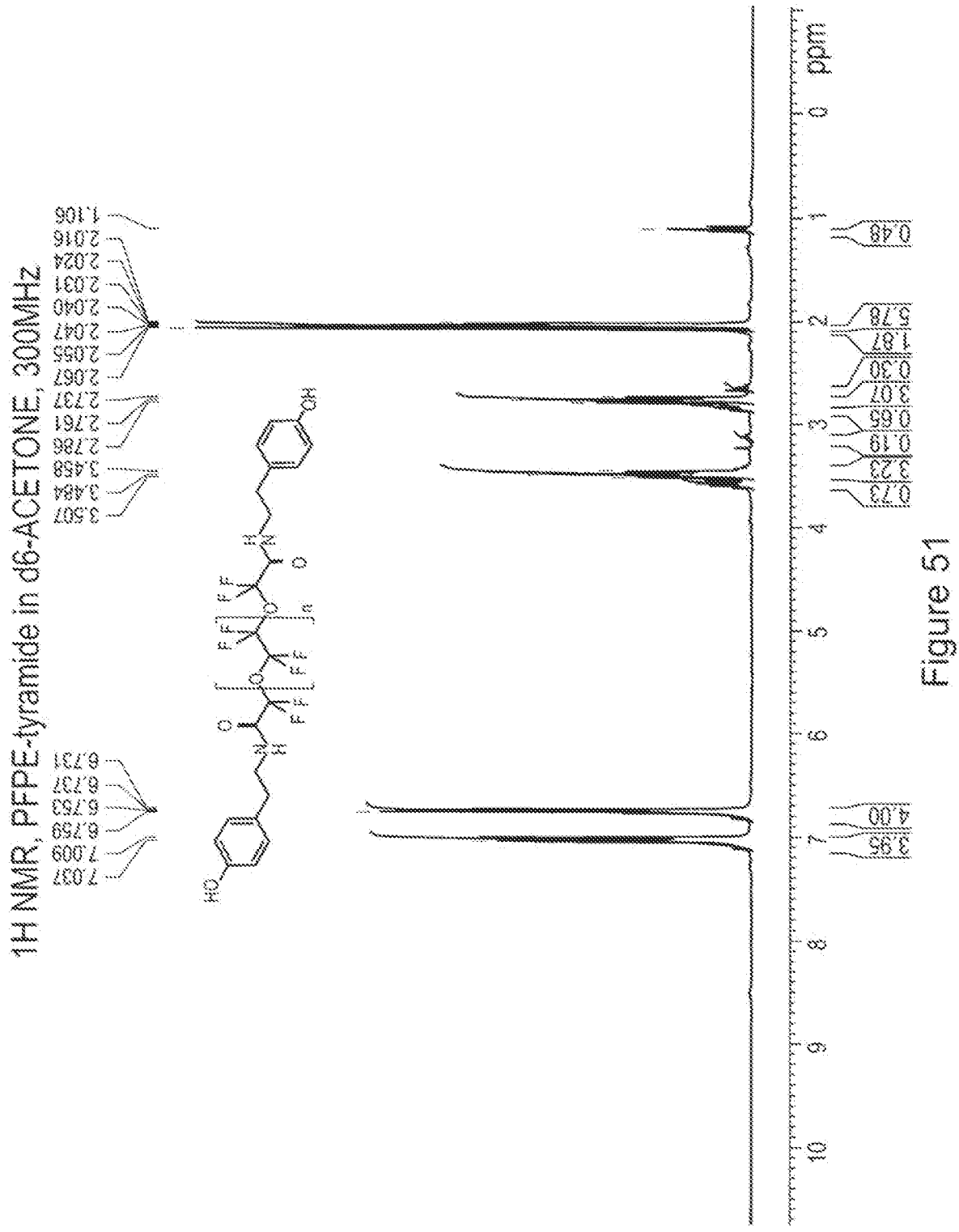

1H NMR, PFPE-tyramide in d6-ACETONE, 300MHz
1.106
2.016
2.024
2.031
2.040
2.047
2.055
2.067
2.737
2.761
2.786
3.458
3.484
3.507
6.731
6.737
6.753
6.759
7.009
7.037
ppm
0.48
5.78
1.87
0.30
3.07
0.65
0.19
3.23
0.73
3.95
4.00

Figure 51

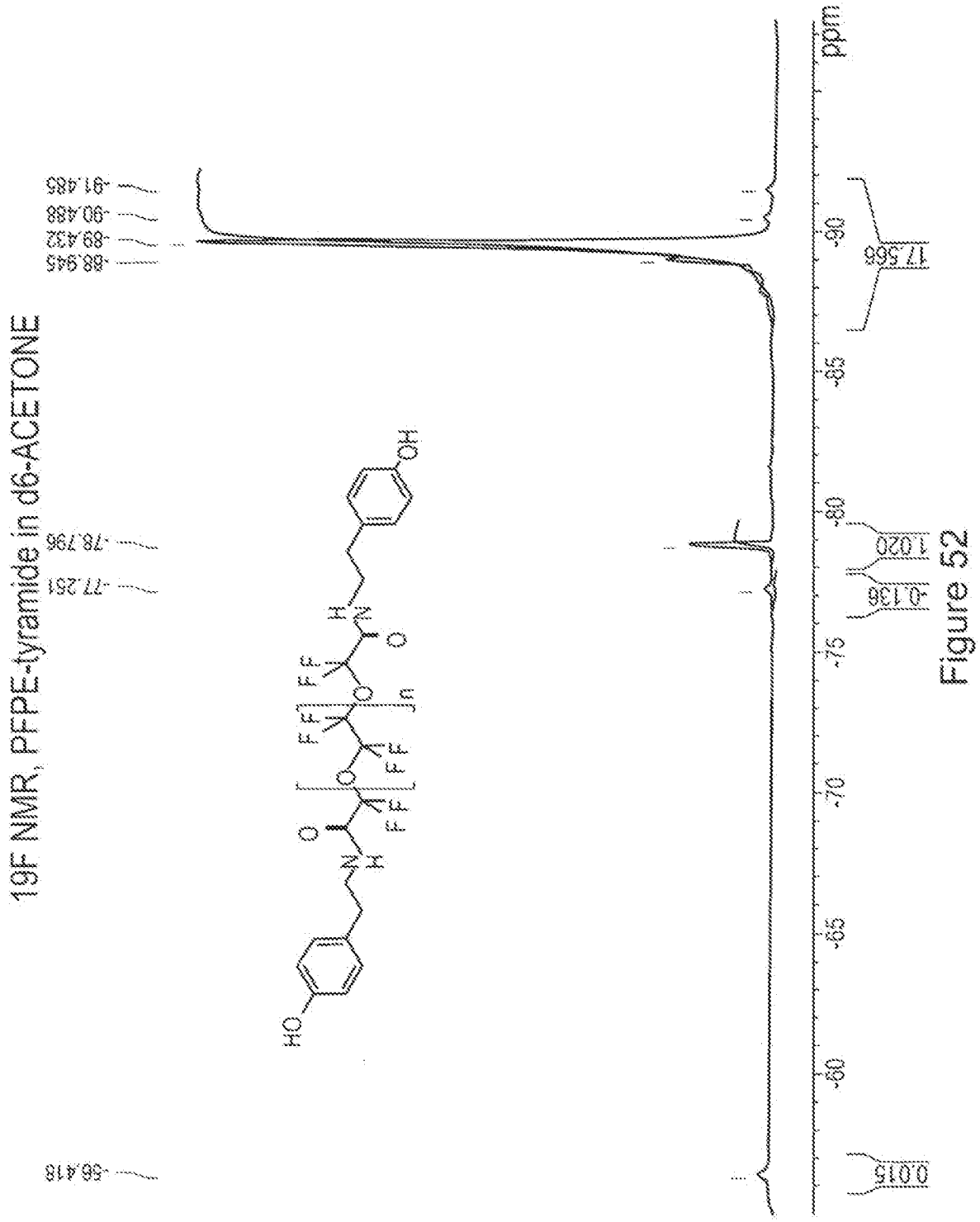

19F NMR, PFPE-tyramide in d6-ACETONE
-91.485
-90.488
-89.432
-88.945
-78.796
-77.251
-56.418
ppm
-90
-85
-80
-75
-70
-65
-60
17.566
1.020
-0.136
0.015
HO
OH
O
N
H
F
n

Figure 52

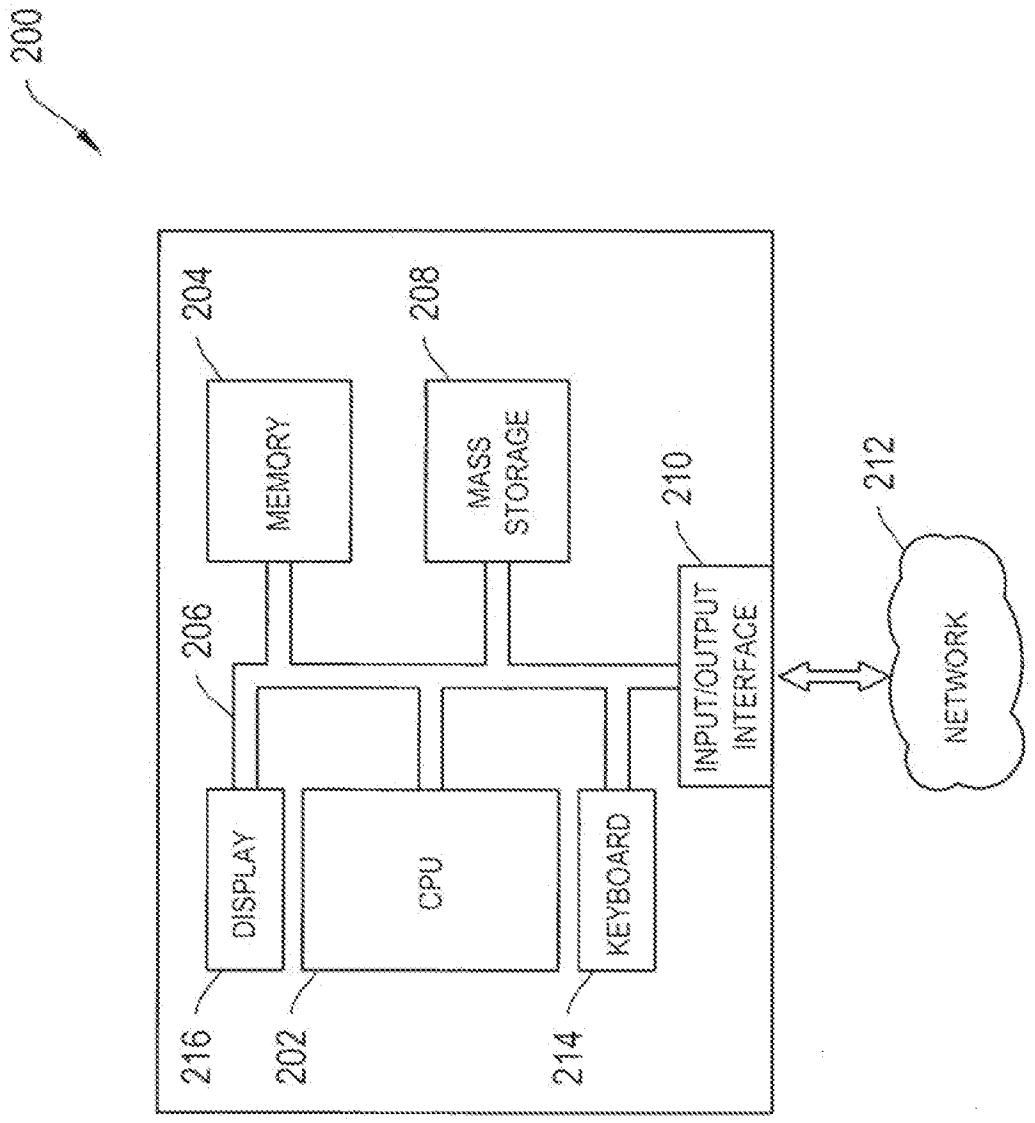

200
204
MEMORY
208
MASS STORAGE
210
INPUT/OUTPUT INTERFACE
212
NETWORK
206
DISPLAY
CPU
KEYBOARD
216
202
214

Figure 53

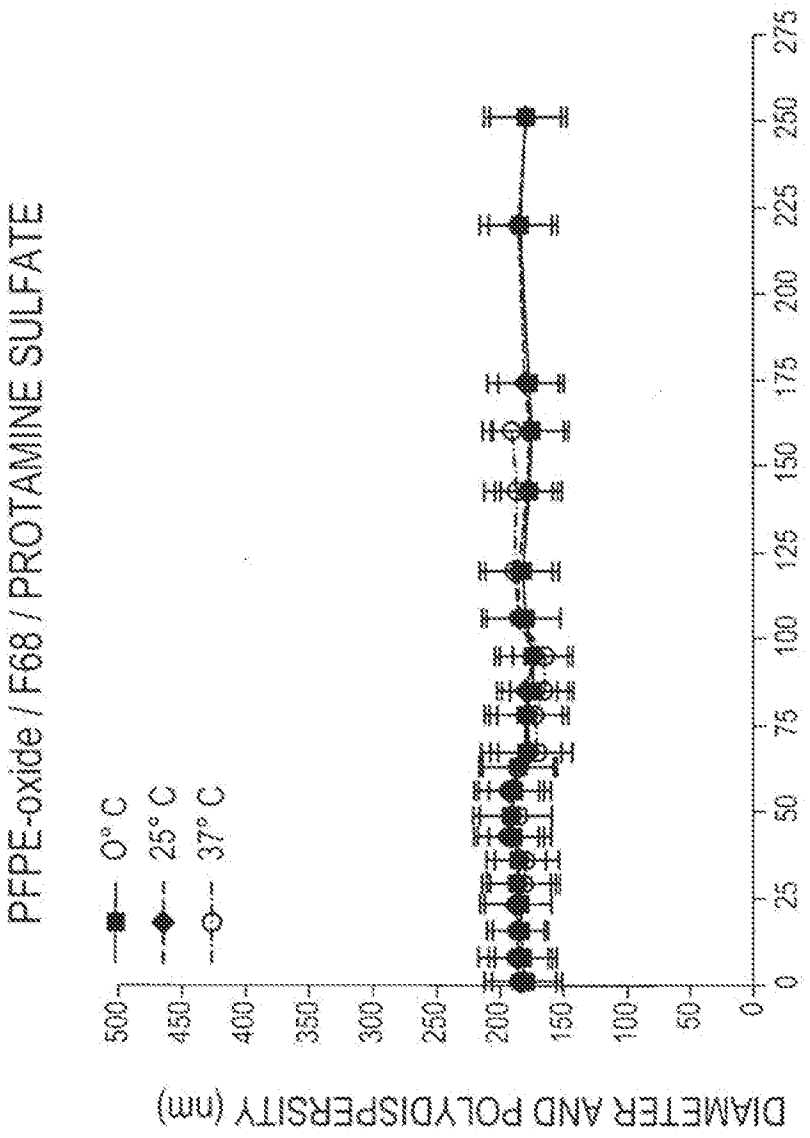

PFPE-oxide / F68 / PROTAMINE SULFATE
0° C
25° C
37° C
DIAMETER AND POLYDISPERSITY (nm)
500
450
400
350
300
250
200
150
100
50
0
0
25
50
75
100
125
150
175
200
225
250
275
DAYS

Figure 54

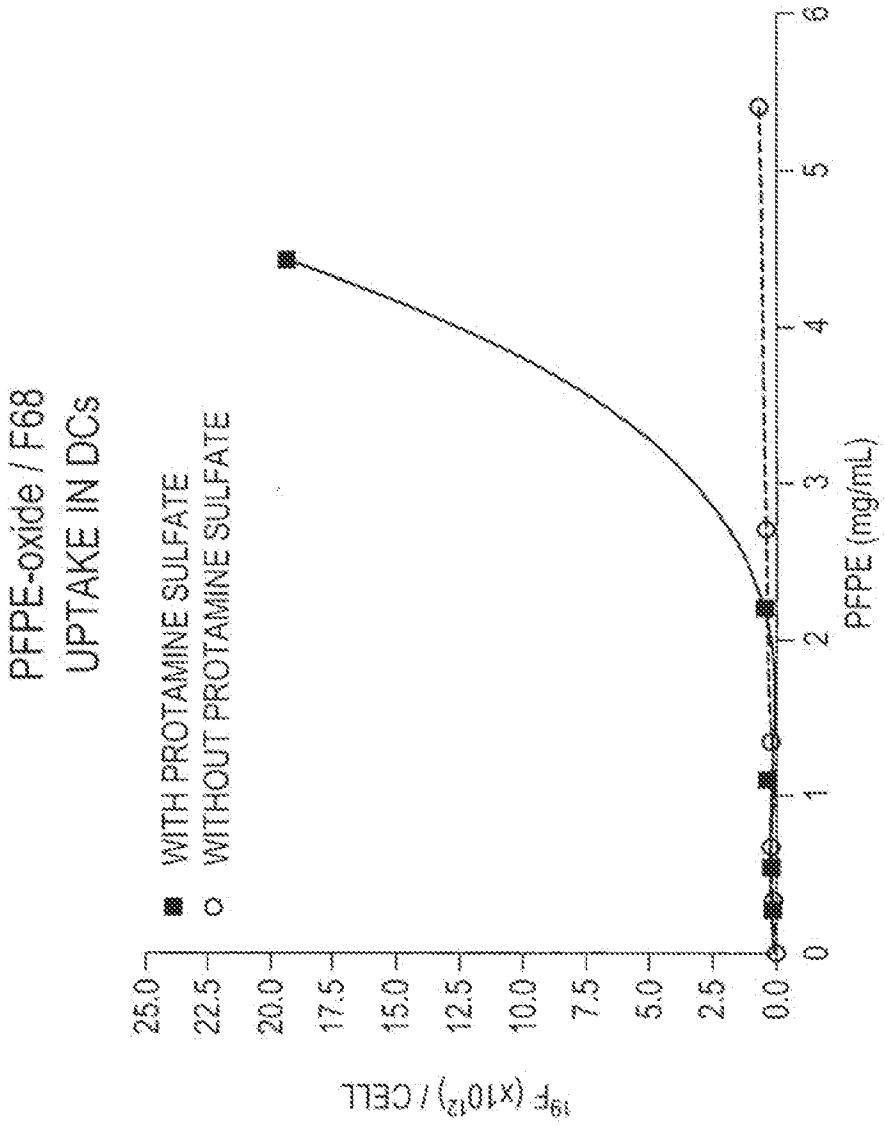

PFPE-oxide / F68
UPTAKE IN DCs
WITH PROTAMINE SULFATE
WITHOUT PROTAMINE SULFATE
^{19}F ($X10^{12}$) / CELL
25.0
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0
1
2
3
4
5
6
PFPE (mg/mL)

Figure 55

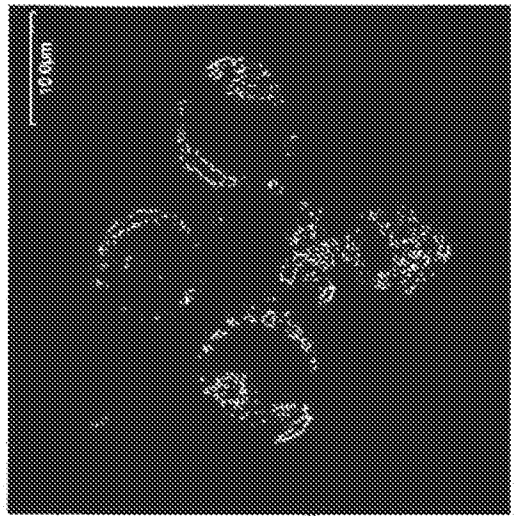


Figure 56C

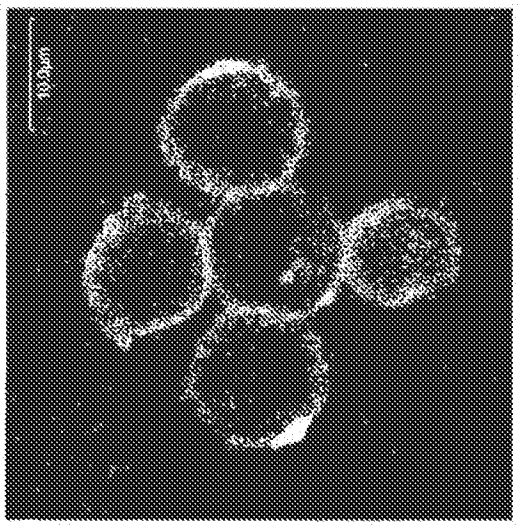


Figure 56B

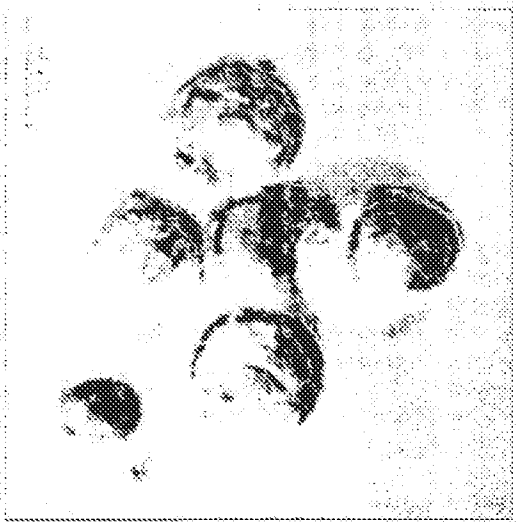


Figure 56A

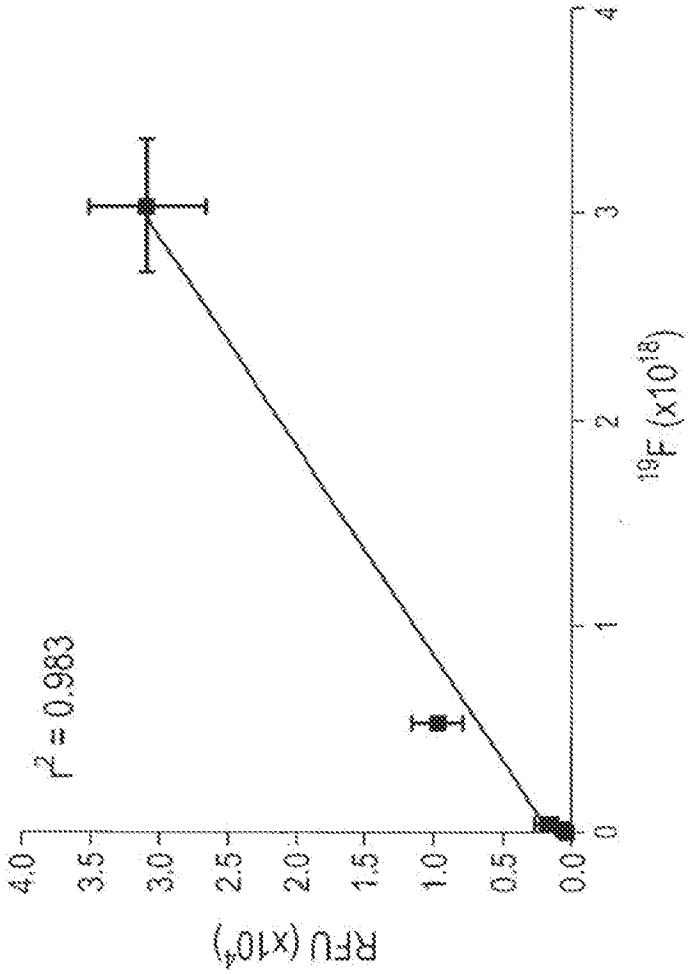


Figure 57

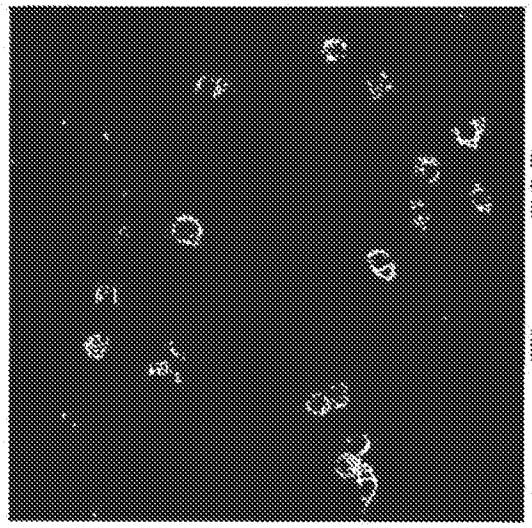


Figure 58B

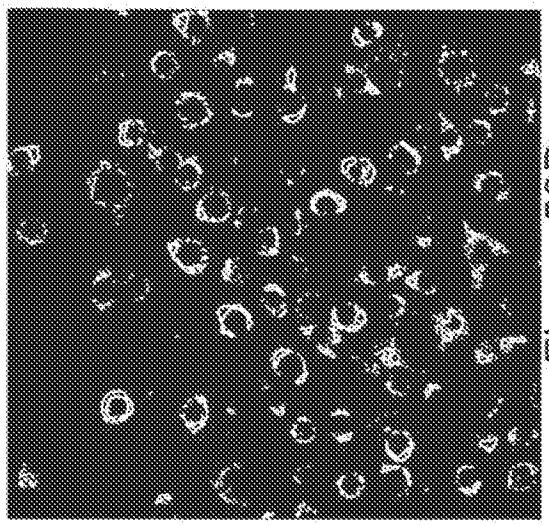


Figure 58D

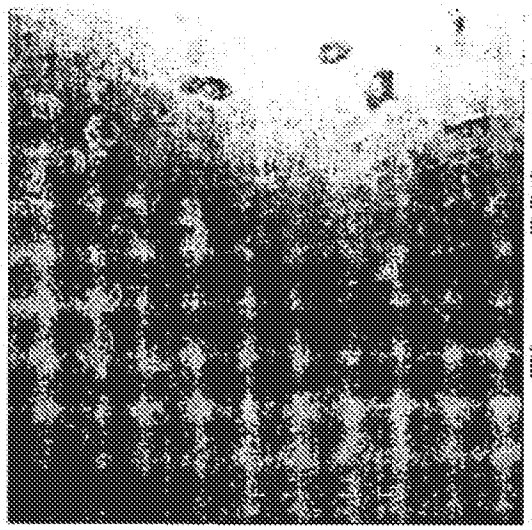


Figure 58A

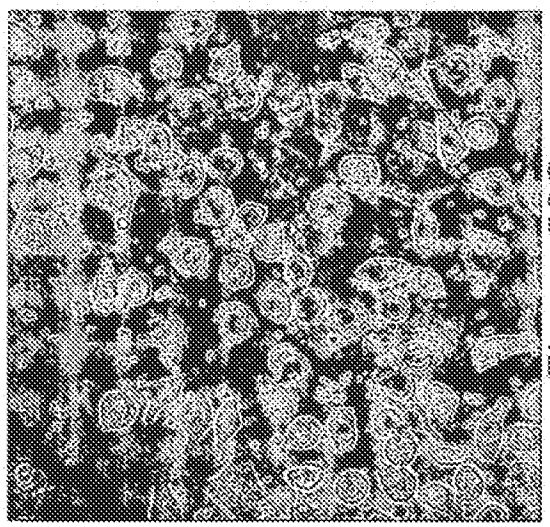


Figure 58C

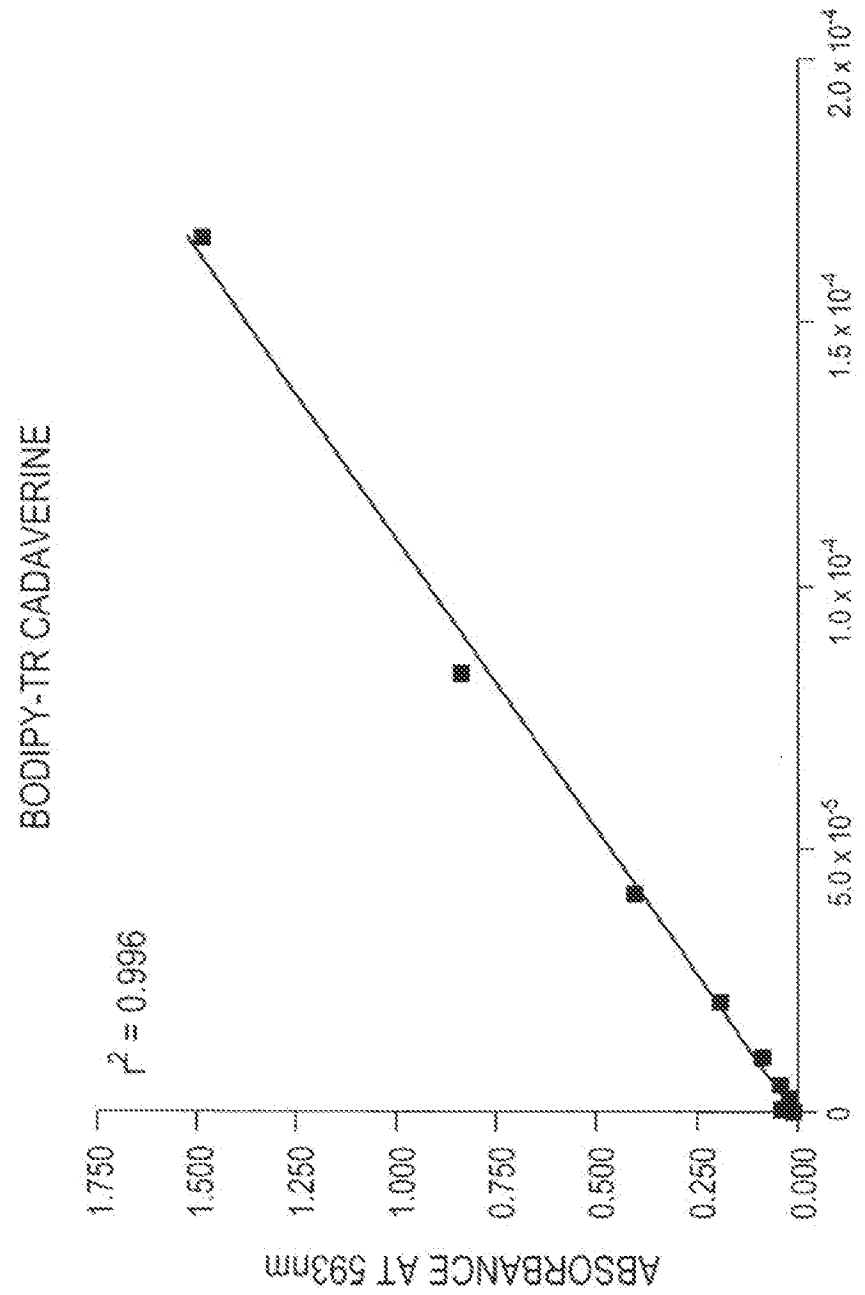

BODIPY-TR CADAVERINE
r^2 = 0.996
ABSORBANCE AT 593nm
1.750
1.500
1.250
1.000
0.750
0.500
0.250
0.000
0
5.0 x 10^{-5}
1.0 x 10^{-4}
1.5 x 10^{-4}
2.0 x 10^{-4}
[M]

Figure 59

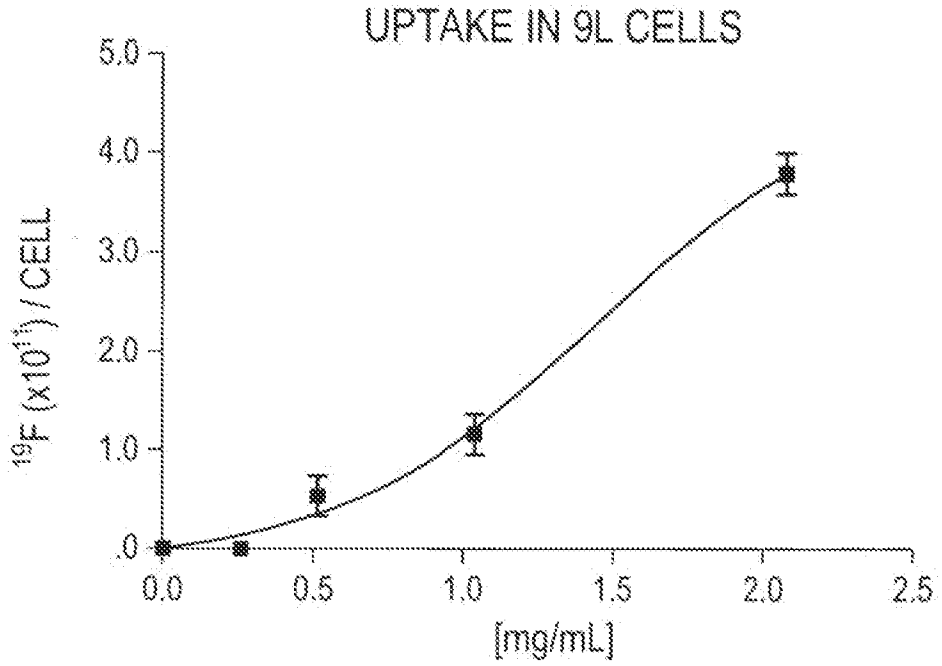
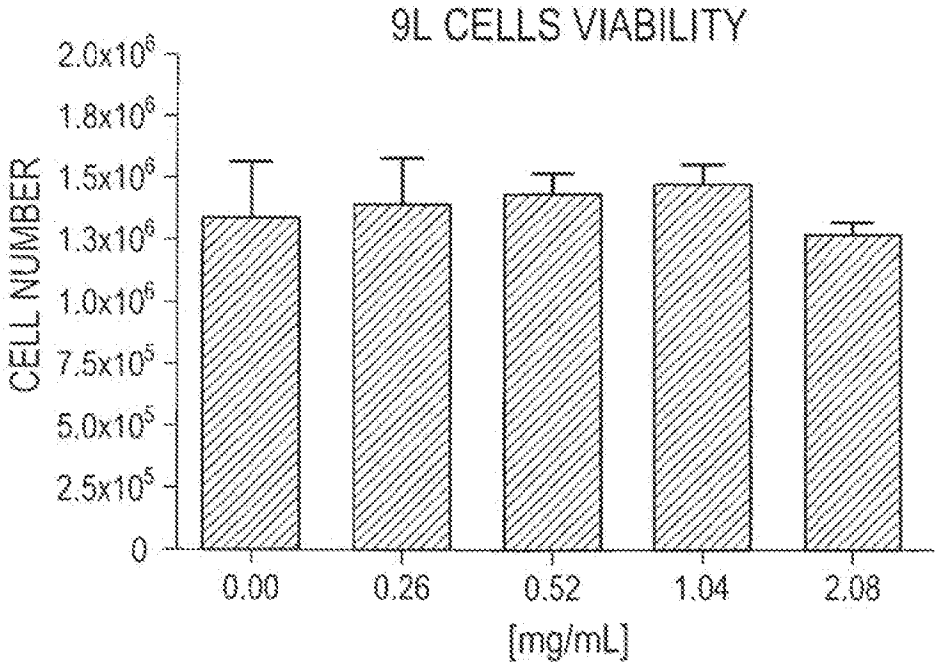

UPTAKE IN 9L CELLS
5.0
4.0
3.0
2.0
1.0
.0
^{19}F (x10^{13}) / CELL
0.0
0.5
1.0
1.5
2.0
2.5
[mg/mL]

Figure 60A


9L CELLS VIABILITY
2.0x10^6
1.8x10^6
1.5x10^6
1.3x10^6
1.0x10^6
7.5x10^5
5.0x10^5
2.5x10^5
0
CELL NUMBER
0.00
0.26
0.52
1.04
2.08
[mg/mL]

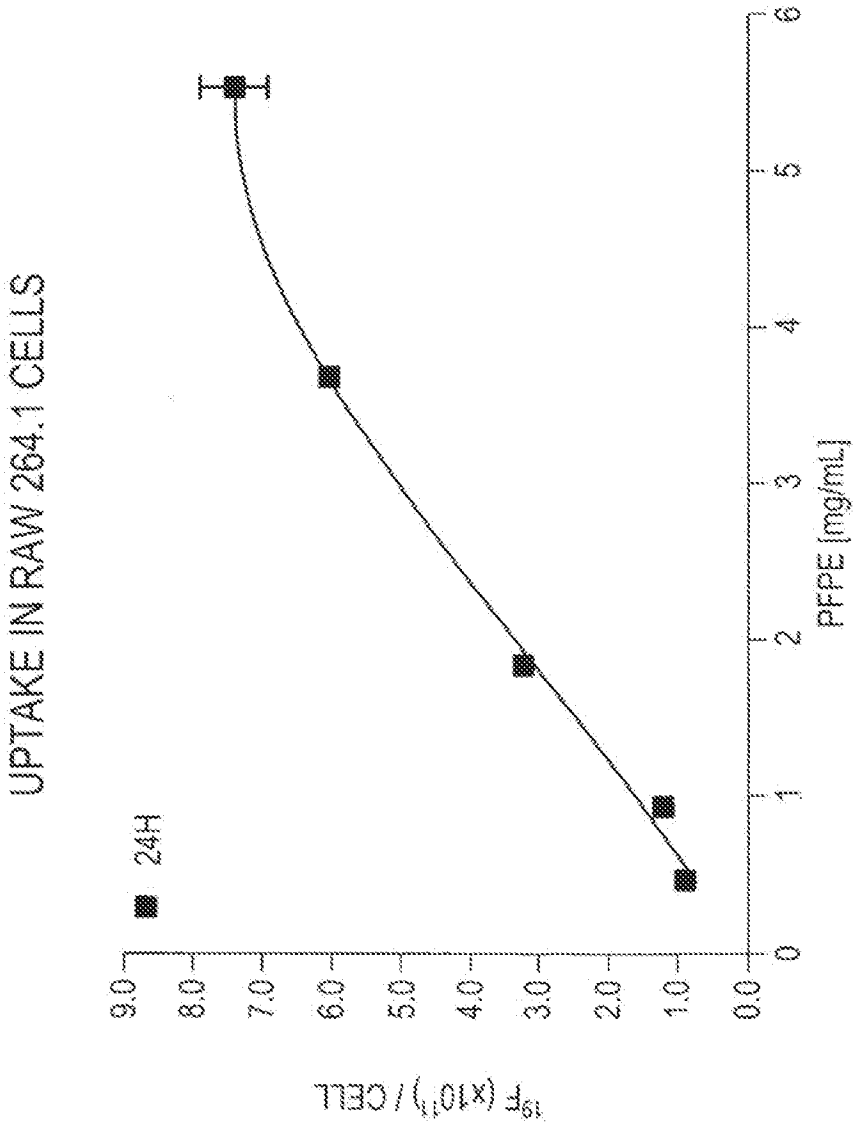

UPTAKE IN RAW 264.1 CELLS
24H
^{19}F ($\times 10^{11}$) / CELL
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
1
2
3
4
5
6
PFPE [mg/mL]

Figure 61

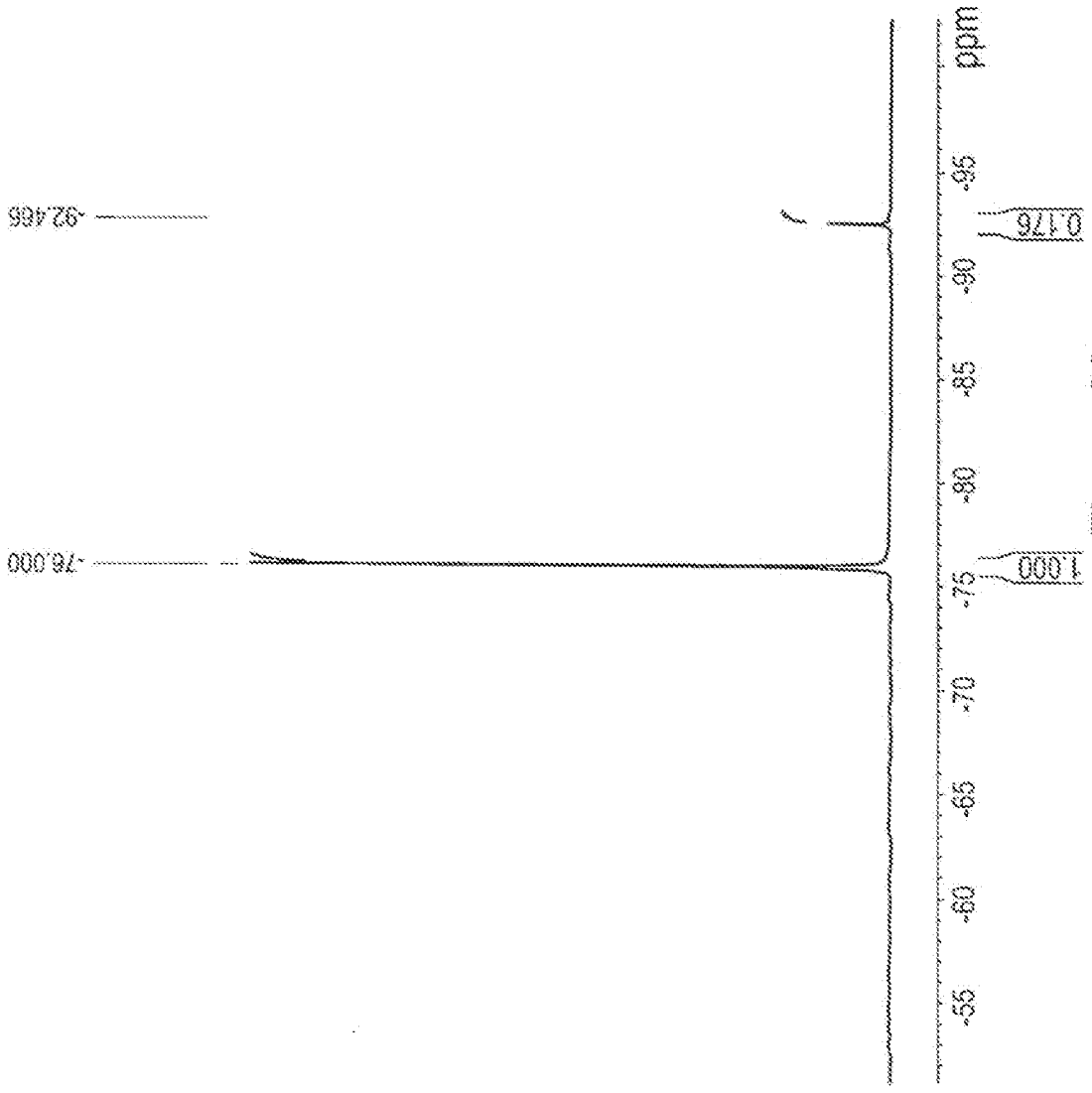


Figure 62

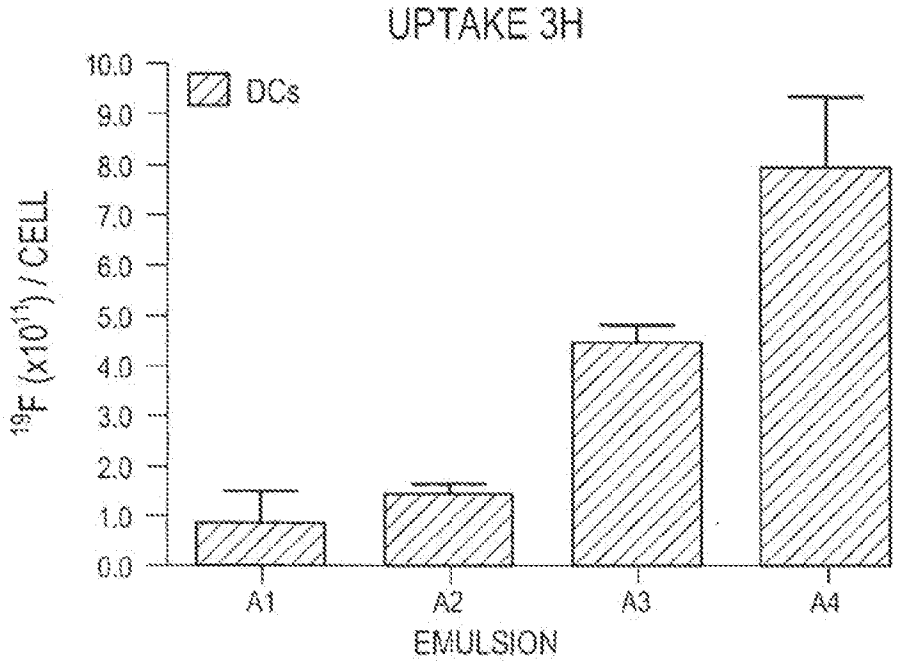


Figure 63A

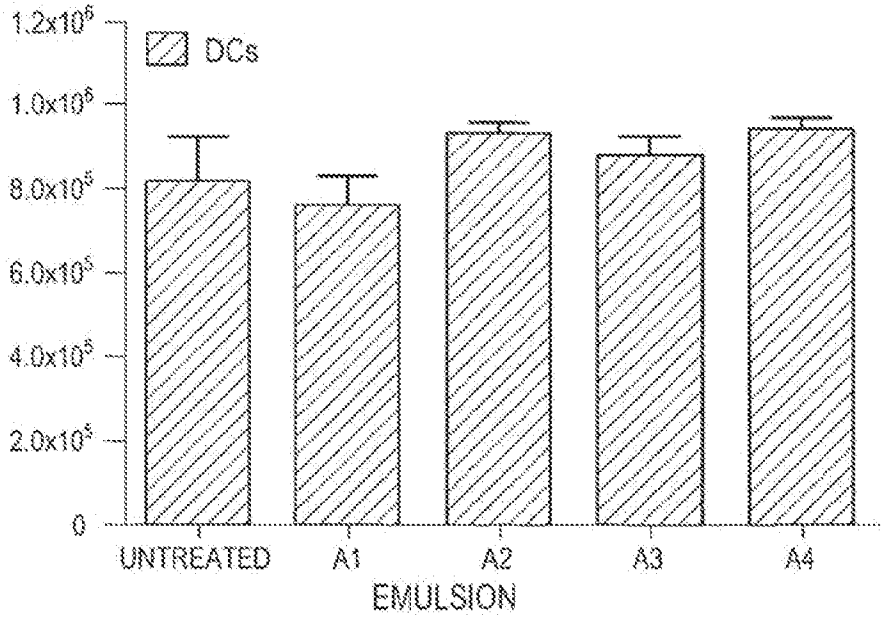


Figure 63B

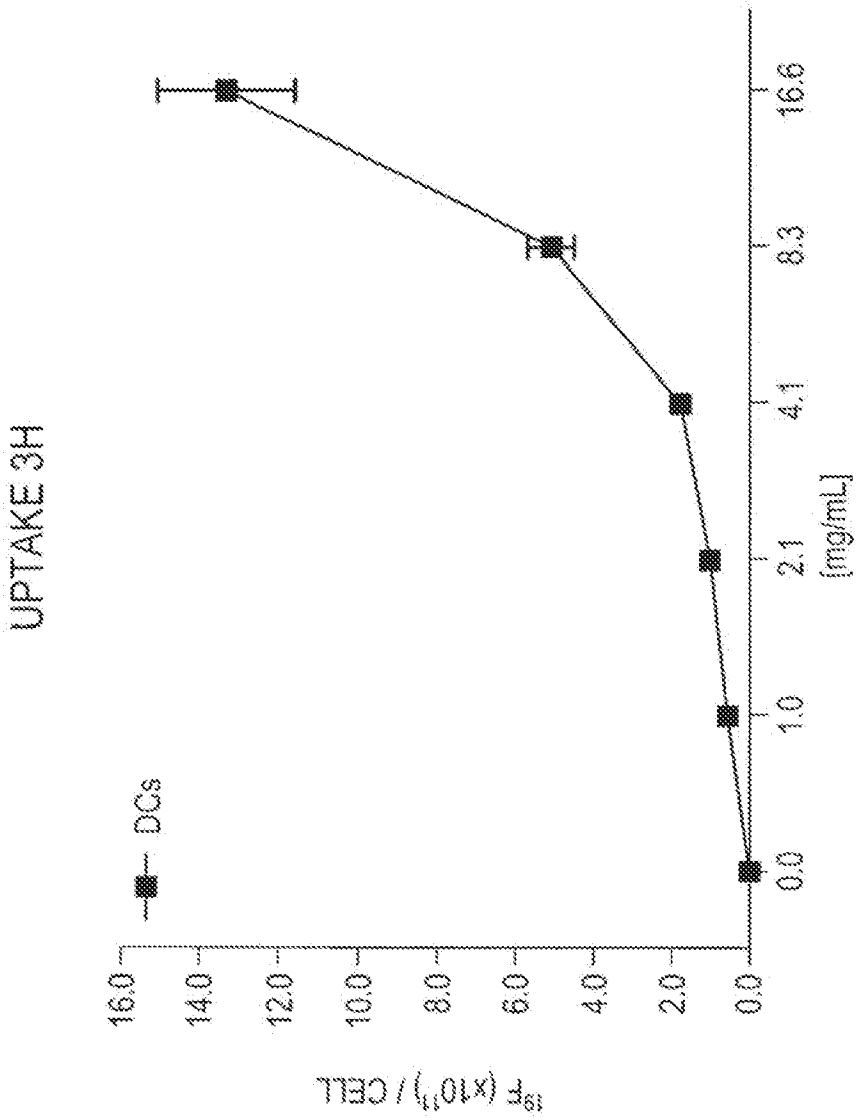

UPTAKE 3H
DCs
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.0
1.0
2.1
4.1
8.3
16.6
[mg/mL]
^{19}F ($\times 10^{11}$) / CELL

Figure 64

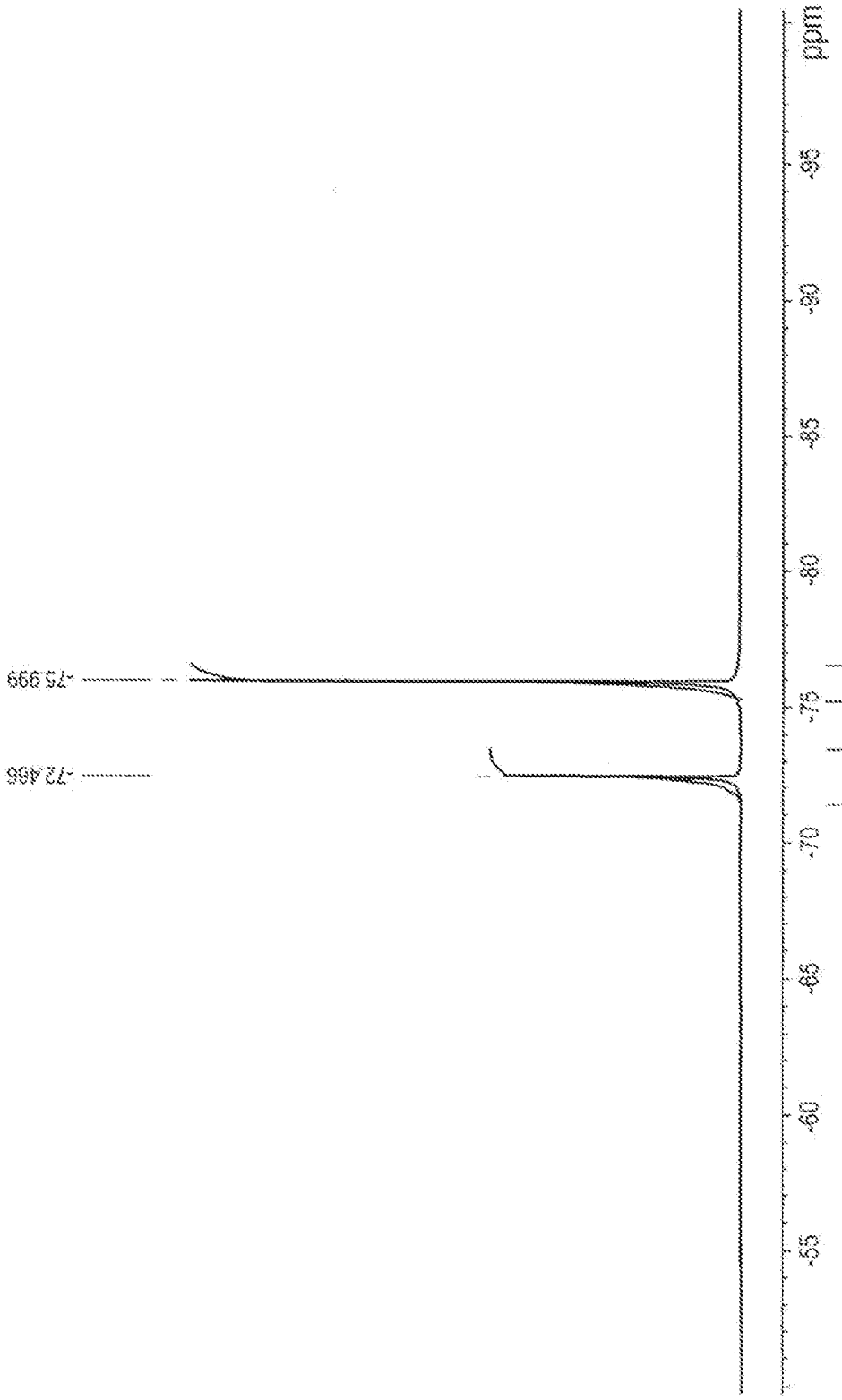


Figure 65

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(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **JANJIC, Jelena** [RS/US]; 181 Gordon Street, Pittsburgh, PA 15218 (US). **AHRENS, Eric, T.** [US/US]; 327 Dewey Street, Pittsburgh, PA 15218 (US).

(74) Agents: **VARMA, Anita** et al.; Ropes & Gray LLP, One International Place, Boston, MA 02110 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
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(54) Title: COMPOSITIONS AND METHODS FOR PRODUCING CELLULAR LABELS FOR NUCLEAR MAGNETIC RESONANCE TECHNIQUES

(57) Abstract: The disclosure provides, in part, compositions and methods for producing cellular labels for tracking cells by MRI. The disclosure provides, in part, methods for labeling, detecting and quantifying cell numbers in vivo.

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INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. C08G65/32 C08G65/48 C08G65/333

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/072780 A (UNIV CARNEGIE MELLON [US]; AHRENS ERIC T [US]) 11 August 2005 (2005-08-11) cited in the application	1
A	abstract; claims 1,17,21,25,32,45,57; figure 12 pages 44-45	16-20, 22,26, 30,34, 38,42, 45-48, 50-71

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 2008	20. 01. 2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schütte, Maya

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/008486

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 728 788 A (SHINETSU CHEMICAL CO [JP]) 6 December 2006 (2006-12-06)	1
A	claims 1-5,11	16-20, 22,26, 30,34, 38,42, 45-48, 50-71

X	US 4 094 911 A (ZOLLINGER JOSEPH LAMAR [US] ET AL) 13 June 1978 (1978-06-13) abstract; claim 1; example I	1
A	Entries 3 and 4 of Table 1; Formulae V, VI, VII column 11, lines 30-53	16-20, 22,26, 30,34, 38,42, 45-48, 50-71

A	SOLOSKI E J ET AL: "SYNTHESIS OF PERFLUORO(POLYETHER) DIFUNCTIONAL COMPOUNDS" JOURNAL OF FLUORINE CHEMISTRY, ELSEVIER, NL, vol. 11, 1 January 1978 (1978-01-01), pages 601-612, XP000577819 ISSN: 0022-1139 Scheme (9), Formulae (XIV) and (XVI)	1,16-20, 22,26, 30,34, 38,42, 45-48, 50-71

A	F. PIACENTI, M. CAMAITI: "Synthesis and characterization of fluorinated polyetheric amides" JOURNAL OF FLUORINE CHEMISTRY, vol. 68, 1994, pages 227-235, XP002500730 cited in the application page 229, column 1, paragraph 3 page 229, column 2, paragraph 5	1,16-20, 22,26, 30,34, 38,42, 45-48, 50-71

P,X	J. M. JANJIC, N. SRINIVAS, D. K. K. KADAYAKKARA, E. T. AHRENS: "Self-delivering Nanoemulisons for Dual Fluorine-19 MRI and Fluorescence Detection" JOURNAL OF THE AMERCAN CHEMICAL SOCIETY, vol. 130, 12 February 2008 (2008-02-12), pages 2832-1841, XP002500933 page 2833, columns 1-2 Scheme 1	1,45-48, 50-63, 65-71

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/008486

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1,16-20,22,26,30,34,38,42,45-48,50-71(as far as compound (1...

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1,16-20,22,26,30,34,38,42,45-48,50-71 (as far as compound (1) is concerned)

Compound according to formula (1) and compositions, emulsions, methods using compound (1)

---

2. claims: 1,45-48,50-63,65-71 (as far as ccompound (2) is concerned)

Compound according to formula (2) and compositions, emulsions, methods using compound (2)

---

3. claims: 1, 45-48,50-63,65-71 (as far as compound (6) is concerned)

Compound according to formula (6) and compositions, emulsions, methods using compound (6)

---

4. claims: 1, 45-48,50-63,65-71 (as far as compound (7) is concerned)

Compound according to formula (7) and compositions, emulsions, methods using compound (7)

---

5. claims: 1, 45-48,50-63,65-71 (as far as compound (8) is concerned)

Compound according to formula (8) and compositions, emulsions, methods using compound (8)

---

6. claims: 2,16,21,22,29,30, 45-48,50-63,65-71 (as far as compound (10) is concerned)

Compound according to formula (10) and compositions, emulsions, methods using compound (10)

---

7. claims: 2, 23,24,31,32,39,40,45-48,50-63,65-71 (as far as compound (11) is concerned)

Compound according to formula (11) and compositions, emulsions, methods using compound (11)

---

8. claims: 2,17,25,26,33,34,41,42, 45-48,50-63,65-71 (as far as compound (12) is concerned)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Compound according to formula (12) and compositions, emulsions, methods using compound (12)

---

9. claims: 2, 27,28,35,36,43-48,50-63,65-71 (as far as compound (13) is concerned)

Compound according to formula (13) and compositions, emulsions, methods using compound (13)

---

10. claims: 2, 18,45-48,50-63,65-71 (as far as compound (14) is concerned)

Compound according to formula (14) and compositions, emulsions, methods using compound (14)

---

11. claims: 2, 45-48,50-63,65-71 (as far as compound (15) is concerned)

Compound according to formula (15) and compositions, emulsions, methods using compound (15)

---

12. claims: 3,19,45-48,50-71 (as far as compound (16) is concerned)

Compound according to formula (16) and compositions, emulsions, methods using compound (16)

---

13. claims: 3,19,45-48,50-71 (as far as compound (17) is concerned)

Compound according to formula (17) and compositions, emulsions, methods using compound (17)

---

14. claims: 3,45-48,50-71 (as far as compound (40) is concerned)

Compound according to formula (40) and compositions, emulsions, methods using compound (40)

---

15. claims: 3,45-48,50-71 (as far as compound (41) is concerned)

Compound according to formula (41) and compositions, emulsions, methods using compound (41)

---

16. claims: 4,5,21,22,29,30,45-48,50-63,65-71 (as far as compounds (26) and (20) are concerned)

Compounds according to formulae (26) or (20)and compositions, emulsions, methods using compounds (26) or (20)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

---

17. claims: 6,7, 23,45-48,50-63,65-71 (as far as compounds (27) and (21) are concerned)

Compounds according to formulae (27) or (21) and compositions, emulsions, methods using compounds (27) or (21)

---

18. claims: 8,32,45-48,50-63,65-71 (as far as compound (28) is concerned)

Compound according to formula (28) and compositions, emulsions, methods using compound (28)

---

19. claims: 9,10,25,26,45-48,50-63,65-71 (as far as compounds (29) or (23) are concerned)

Compounds according to formulae (29) or (23) and compositions, emulsions, methods using compounds (29) or (23)

---

20. claims: 11,12, 27,35,45-48,50-63,65-71 (as far as compounds (30) or (24) are concerned)

Compounds according to formulae (30) or (24) and compositions, emulsions, methods using compounds (30) or (24)

---

21. claims: 13,14, 28,36,45-48,50-63,65-71 (as far as compounds (31) or (25) are concerned)

Compounds according to formulae (31) or (25) and compositions, emulsions, methods using compounds (31) or (25)

---

22. claims: 15,37,38,45-48,50-63,65-71 (as far as compound (32) is concerned)

Compound according to formula (32) and compositions, emulsions, methods using compound (32)

---

23. claims: 15,39,45-48,50-63,65-71 (as far as compound (33) is concerned)

Compound according to formula (33) and compositions, emulsions, methods using compound (33)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

24. claims: 15,40,45-48,50-63,65-71 (as far as compound (34) is concerned)

Compound according to formula (34) and compositions, emulsions, methods using compound (34)

---

25. claims: 15,41,42,45-48,50-63,65-71 (as far as compound (35) is concerned)

Compound according to formula (35) and compositions, emulsions, methods using compound (35)

---

26. claims: 15,43,45-48,50-63,65-71 (as far as compound (36) is concerned)

Compound according to formula (36) and compositions, emulsions, methods using compound (36)

---

27. claims: 15,44,45-48,50-63,65-71 (as far as compound (37) is concerned)

Compound according to formula (37) and compositions, emulsions, methods using compound (37)

---

28. claims: 45,46,50-64,66-71 (as far as compound (1a) is concerned)

mixtures and emulsions comprising compound according to formula (1a)

---

29. claims: 47,48,50-64,66-71 (as far as perfluoro-15-crown-5 ether is concerned)

mixtures and emulsions comprising perfluoro-15-crown-5 ether

---

30. claims: 49-64,66-71 (as far as compound (42) is concerned)

Compound according to formula (42) and compositions, emulsions, methods using compound (42)

---

31. claims: 72-78

methods for labelling, detecting, quantifying cells and a labeled cellular formulation

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/008486

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005072780 A	11-08-2005	AU 2005209169 A1	11-08-2005
		CA 2560544 A1	11-08-2005
		EP 1713513 A2	25-10-2006
		JP 2007526245 T	13-09-2007
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EP 1728788 A	06-12-2006	JP 2006335955 A	14-12-2006
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US 4094911 A	13-06-1978	NONE	